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Dinitrogen (C₂H₂) fixation, total plant yield, and total nitrogen percentage in alfalfa (*Medicago sativa* L) as affected by plant density, cutting management, and cultivar

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DINITROGEN (C₂H₂) FIXATION, TOTAL PLANT YIELD, AND TOTAL
NITROGEN PERCENTAGE IN ALFALFA (MEDICAGO SATIVA L.) AS
AFFECTED BY PLANT DENSITY, CUTTING MANAGEMENT, AND
CULTIVAR

Iowa State University

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nitrogen percentage in alfalfa (Medicago sativa L.)
as affected by plant density, cutting
management, and cultivar

by

William H. Bohl

A Dissertation Submitted to the
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Iowa State University
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1981

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CHAPTER I. INTRODUCTION

Recommended current practices in alfalfa (Medicago sativa L.) production are geared to maximize yield and quality of forage. Cultivars available and cultural practices, however, which are associated with maximizing yield and quality may not necessarily result in maximum dinitrogen fixation rates (Vance, 1978). This is so because plant density, soil fertility, and cutting management along with other factors all may vary.

The importance of alfalfa as a source of nitrogen was recognized many years ago (Lyon and Bizzell, 1934), yet it is necessary to once again stress that we should increase our dependence on biologically fixed nitrogen (Evans and Barber, 1977). Alfalfa fixes from 50 to 75% of the total nitrogen in the plant with the remainder coming from soil nitrogen (Vance, 1979). It is known that nodulation and nitrogen fixation can be impaired by such factors as soil acidity, incorrect temperature, and imbalanced soil fertility (Lie, 1971; Silver and Hardy, 1975). Little is known about the effect of cultural practices on the rate of dinitrogen fixation in alfalfa.

With the ever-increasing scarcity of fossil fuels and the demand for nitrogen fertilizer increasing, it is evident that increases in use of a nitrogen-fixing crop such as alfalfa could be important. It is necessary to reevaluate, therefore, alfalfa's contribution under modern technology.

We questioned the current practices geared to maximizing dry matter yields to the extent that these same practices may or may not result in maximum rates of dinitrogen fixation.

The objective of this study was to determine how plant density, cutting management, and cultivar affects the rate of dinitrogen fixation in alfalfa. Another objective of this study was to determine the forage and root dry matter yield, percentage forage and root nitrogen, nitrogen removed in the forage, and root nitrogen accumulation as affected by the same treatments. Throughout the remainder of this dissertation, the term nitrogen and dinitrogen fixation will be used synonymously.

CHAPTER II. REVIEW OF LITERATURE

Introduction

There have been numerous nitrogen fixation studies conducted on such plant species as field beans (Phaseolus vulgaris L.) (Graham and Rosas, 1978), field peas (Pisum sativum L.) (Lawrie and Wheeler, 1973), and soybeans (Glycine max (L.) Merr.) (Wahua and Miller, 1978; Trang and Giddens, 1980). Little research has been done, however, using forage legumes. Of the experiments on nitrogen fixation in forage legumes, a preponderance is on white clover (Trifolium repens L.) (Moustafa et al., 1969; Butler et al., 1959; Chu and Robertson, 1974; Masterson and Murphy, 1976), red clover (Trifolium pratense L.) (Butler et al., 1959), or other forage legumes (Day and Dart, 1969a). While it may be possible to relate the results of these studies using other forage or grain legumes to what may happen in alfalfa if grown under similar conditions, the fact remains that experimental evidence on nitrogen fixation in alfalfa is limited.

Even though this study was concerned only with plant density, cutting management, and alfalfa cultivar, a review of other factors affecting growth is presented. These are: shading within the canopy, water availability, temperature conditions, and others. Such findings are deemed pertinent to the interpretation of results of this study.

Methods of Studying Nitrogen Fixation

Burns and Hardy (1975) discuss several methods for studying the rate of nitrogen fixation. Of these, the use of $^{15}\text{N}_2$ has proven to be a very sensitive method offering about 1000-fold more accuracy than that of the Kjeldahl method. Though the method is accurate, two disadvantages are the cost of the material and the destruction of the sample in the analysis.

In the mid-1960s, researchers used acetylene to inhibit the reduction of atmospheric nitrogen. Reduction of acetylene to ethylene is an indication of nitrogenous activity (Burns and Hardy, 1975). Acetylene reduction to study nitrogen fixation has been used extensively by many researchers. Burns and Hardy (1975) list several advantages of this method: (1) more sensitive than $^{15}\text{N}_2$, (2) requires rather simple equipment such as a gas chromatograph, (3) the product ethylene is easily detected, (4) it is very rapid, (5) ethylene can be stored indefinitely in gas-tight containers before analysis, and (6) samples need not be destroyed for analysis.

Several authors have found the acetylene reduction method to be a reliable method of studying nitrogen fixation (Hardy et al., 1968; Haystead and Lowe, 1977; Sinclair, 1975). The assay may be conducted using detached nodules (Bergersen, 1970; Mague and Burris, 1972), intact plants (Krall and

Delaney, 1979; Mederski and Streeter, 1977), plants growing in potted soil (Sinclair, 1973; Sinclair et al., 1978), or roots contained in soil cores (Sinclair et al., 1976).

Sinclair (1973) studied the validity of using the acetylene reduction method on plants growing in soil. White clover plants were grown in pots in either a loamy sand soil or silica sand. Ten pots of each type of soil were used. After 10 weeks all plots with growing plants were analyzed for ethylene production by placing the entire pot in an incubation container for 6 hours. On the basis of results from this analysis, pots of plants were divided into pairs of equal acetylene reduction rates. Two days later, plants from one of each pair of pots were removed from the pot, soil was carefully removed, and acetylene reduction assays were performed on each of the pairs of plants. No differences were reported in the rate of acetylene reduction of plants growing in soil or whether the root system with the nodules had been exposed to the acetylene.

In a later study, Sinclair et al. (1976) evaluated the use of acetylene reduction assays in small soil cores with white clover growing therein. They compared the rate of acetylene reduction in 2.54 x 10-cm soil cores with that of 15 x 15 x 10-cm white clover-ryegrass pasture turfs and found that within the first 2 hours both types of samples gave similar results. After 6 hours, however, the soil cores produced values less than the turf sections. In the same study, over 80% of

the acetylene reduction activity was determined to be in the surface 7.5 cm for alfalfa, red clover, white clover, and alsike clover (Trifolium hybridum L.).

Even though the acetylene reduction assay is accurate, it has been emphasized that large errors may result if the experimental conditions are not carefully controlled and if a large enough sample size is not taken (Bergersen, 1970; Sinclair et al., 1976). It is not uncommon to have coefficients of variation of 25% (Sinclair et al., 1976).

Management Effects on Nitrogen Fixation

Three management practices will be discussed in relation to the effect on nitrogen fixation or yield. All three, i.e., cutting management, plant density, and cultivar, can be controlled by the producer and researcher of alfalfa.

Cutting management

In considering the life cycle of plants, one of the most severe agronomic treatments imposed on plants probably is the complete removal of top growth. This is frequently done on perennial forage species such as alfalfa.

In one of the earlier studies of effects of defoliation on nitrogen fixation in white clover, Wilson (1942) studied the loss of nodules resulting from cutting. Greenhouse-grown plants were cut to 2.54 cm after reaching a height of 10 cm. Results showed that cutting caused a loss of nodules

and that two cuttings caused a more severe loss than did a single cutting. Before the first cutting treatment, most of the nodules were located in the surface 2.54 cm. After cutting, however, these nodules were replaced by new ones located on the tap root as well as by nodules on the lateral root branches farther below the soil surface.

Langille and Calder (1971) studied birdsfoot trefoil (Lotus corniculatus L.). When harvested at a height of 7.5 cm rather than at 2.5 cm, there were significantly more nodules per plant. The effect of cutting height was more pronounced in plants cut four times at the vegetative stage than for plants harvested three times at 10% bloom, twice at 50% bloom, or once at beginning seed production. The number of nodules per plant increased as the frequency of harvests decreased.

In a study on two tropical pasture legumes, greenleaf desmodium (Desmodium intortum (Mill.) Uch.) and siratro (Phaseolus atropurpureus), Whiteman (1970a) stated that:

The weight per nodule declined even though the nodule number increased, and thus the nodule weight per plant increased or remained constant. This provided evidence that changes in nodule weight induced by defoliation were related to a loss of part of the original nodule population and initiation of new nodules.

The roots of a high-yielding clone of alfalfa showed fivefold more and the crowns tenfold more ammonium than was determined to be in the roots and crowns of a low-yielding clone of alfalfa (Chatterton et al., 1977). The low-yielding

clones contained more nitrate-nitrogen in the herbage and roots but less in crowns than the high-yielding clones. Because of the high level of ammonium and low level of nitrate-nitrogen in the roots of the high-yielding clones, these authors suggested a higher nitrogen fixation rate. They reasoned this from the fact that ammonium is a key intermediate in the reduction of atmospheric nitrogen.

Hoglund et al. (1974) cut alfalfa either five times at pre-bud, four times at 50% bud stage, or three times at first flower to study the effects of clipping on nitrogen accumulation and yield. Results indicated that more total dry matter was harvested by cutting at the first flower stage. There was no significant difference resulting from cutting treatments, however, in total herbage nitrogen, as based on Kjeldahl determinations.

Nitrogen fixation rates in white clover were studied by acetylene reduction analysis (Moustafa et al., 1969). Complete defoliation caused a significant reduction in acetylene reduction rates. The rate fell rapidly after cutting and continued to decline until a low was reached on the sixth day. This low remained until the eighth day at which time an increase in acetylene reduction rates was noted. By 34 days after cutting, there was no significant difference in acetylene reduction rates for the cut and uncut plants.

Possible reasons for decreased acetylene reduction activity in white clover after defoliation may include any of

the following: (1) active meristems removed, (2) lack of carbohydrates in the host, or (3) excess of internal nitrogen for current demands (Hoglund and Brock, 1978).

Removal of 70 to 80% of the top growth of alfalfa in the 1% flower stage caused acetylene reduction rates to decline 88% as compared to control plants (Vance et al., 1979). The most rapid decline occurred within 24 hours after harvest and remained at this low level for 13 days before beginning to increase. For the regrowth plants, rate of acetylene reduction was at about the same level as control plants by 18 days after harvest and even exceeded that of controls by day 26. It was observed that no net loss of nodules occurred because of harvests since nodule fresh weight per plant followed the same pattern as root dry weight. There was an inverse relationship observed between acetylene reduction rate and nitrate reductase activity in the harvested plants. Nitrate reductase activity in control plants remained unchanged during the course of the study. From this they concluded that following harvest, alfalfa plants:

...have an adaptive mechanism for continued growth and development. ...and may also have an alternate mechanism for supplying nitrogen to the plant when nitrogen fixation is impaired after harvest.

The exact mechanism employed for nodule regeneration and increased nitrate reductase activity is unclear.

In a greenhouse study, alfalfa was harvested at flowering stage, then regrown for 28 days to 1% flower before being cut

again at approximately 3.5-cm stubble height (Vance et al., 1980). Harvested plants showed senescent nodules within 4 days after clipping. The zone of senescent material increased in size for 10 days following harvest and remained this size from 10 to 18 days after cutting while the zone of viable bacteroids increased in size. Eighteen days after flowering, uncut control plants showed a senescent zone within the nodules.

Plant density

There is little experimental data relative to the effect of plant density on the nitrogen fixation rate in alfalfa or in any of the agronomically important forage legumes. What little research has been done on plant density in forage legumes is mainly concerned with its effect on yield and survival.

Bessac (1968) studied the effects of 14.5- and 29-cm row spacings and seeding rates of 50, 100, or 200 seeds/m of row on the yield and quality of alfalfa. If water was not limiting, the highest yields were recorded at the narrower row spacing. The higher seeding rate resulted in a more favorable winter survival, as compared to the lower rates. Quality factors, which included the percentage nitrogen, were affected little by row spacing or seeding rate.

Eleven alfalfa cultivars varying in winterhardiness were used to study the relationship of yield to plant density and

chemical composition (Porter and Reynolds, 1975). The average plant density in the fall of the seeding year was 55 plants/m². Five harvests were taken each year at the 1/10 bloom stage. Nitrogen percentage was lowest in the June through August period but there was no correlation to yield. When averaged over all cultivars, there was a positive significant correlation between plant density and yield. The moderately nonhardy cultivars produced the lowest yield and had the lowest plant density.

Alfalfa was hand-planted at spacings of 2.54, 5.08, 7.62, 15.24, 22.86, 30.48, 60.96, and 91.44 cm "on the square" (Jarvis, 1962). Yields at the highest four populations were rarely different significantly, but yield decreased as plant population decreased. There were only small differences in nitrogen percentage and significant differences only occasionally resulted at the lower populations. Root yield at the end of 3 years showed no difference resulting from population effects, except at the lowest two, where yields were less than for the other six.

Roufail (1975) reported no significant differences in yield of alfalfa resulting from seeding rate differences which ranged from 6.7 to 26.9 kg/ha under irrigated conditions. Yield was significantly less at a row spacing of 30 or 45 cm, however, as compared to 15 cm. The lower yield for the wider row spacing was thought to result from greater competition among plants. Plant density also had an effect on the

type of root which developed. Plants grown at a density of 75 plants/m² had large tap roots with diameters of 4 cm whereas those in densities of 400 plants/m² had smaller roots with the top of the tap root having only a 1-cm diameter.

Phillips and Bennett (1978) studied the effect of plant density on acetylene reduction rates in subterranean clover (Trifolium subterraneum L.) and soft chess (Bromus mollis L.). Seeding rates were 10, 141, or 1970 seeds/m² in pure stands or in a mixture with equal portions of each. The higher plant densities resulted in more nitrogen per hectare being fixed than at the lower seeding rates. In the pure clover stands, the peak acetylene reduction activity occurred later in the season as the plant density decreased.

Research with soybeans has shown that greater acetylene reduction activity can be obtained at lower plant densities when expressed on a per plant basis, but this activity is unaffected by row width (Hardy et al., 1972). Sprent and Bradford (1977) reported the same effect of row width on broad beans (Vicia faba L.). In this experiment, the higher seeding rates had higher acetylene reduction rates early in the season but by mid-July the lower seeding rates had surpassed the rates of the higher seeding rates. These results were also on a per plant basis.

The rate of ethylene production in field beans (Phaseolus vulgaris L.) was found to be dependent not only on plant density but also on growth habit (Graham and Rosas, 1978). A

highly branched, prostrate, indeterminate cultivar of field bean had consistently higher acetylene reduction rates at all densities studied over the other two cultivars, one an aggressive climber and the other a determinate, early-maturing cultivar. Maximum acetylene reduction rates of all three cultivars on a per unit area was found at the highest seeding rate of 120 plants/m². Nodules were more concentrated on the crown area at higher densities as compared to nodules more localized in secondary roots in the lower plant densities. Nodules at the lower plant densities were larger than those found on plants at higher plant densities. The authors concluded that "the major effect of plant density on fixation per plant was through changes in nodule fresh weight."

Cultivar, genotype, and growth habit

There has been recent interest in increasing nitrogen fixation rates by selecting cultivars that are more efficient in fixing nitrogen. Differences in nitrogen fixation rates due to cultivar have been shown to occur in alfalfa (Seetin and Barnes, 1977), subterranean clover (Nutman, 1961, 1967), crimson clover (Trifolium incarnatum L.) (Smith et al., 1980), white clover (Connolly and O'Keefe, 1979), and red clover (Nutman et al., 1971).

Seetin and Barnes (1977) reported that increased acetylene reduction rates were correlated with an increased number of nodules, increased number of fibrous roots, greater root

weight, and greater top weight in alfalfa. The authors stated that, "based on results from this study, top growth, root morphology, and nodule score are traits which are associated with acetylene reduction in alfalfa."

An experimental strain of alfalfa was found to have some plants which would form nodules, but these nodules would not reduce acetylene to ethylene (Viands et al., 1979). These nonacetylene-reducing plants had larger nodules than plants of the same strain which would reduce acetylene. Three possible explanations for the inability of these plants to reduce acetylene were given: (1) metabolites produced by the host prevent bacteroid development, (2) inability of bacteroids to utilize translocated nutrients, or (3) deficiency of some essential protein.

A semidormant cultivar of alfalfa, 'Mesilla', considered to be a poor nitrogen fixer, was grown in a greenhouse study in pots and analyzed for acetylene reduction rates at 10 weeks (Duhigg et al., 1978). Plants selected for high acetylene reduction activity out of an initial population had a larger number of nodules with darker red color, larger root system, more top dry weight, and more total nitrogen in the top growth than the original cultivar from which they were selected.

Stickler and Johnson (1957) used a nonhardy alfalfa cultivar, 'African', and a hardy cultivar, 'Ranger', to study the effect of cutting on nitrogen production. Nitrogen concentration of the tops and roots was determined by Kjeldahl

analysis after imposing one of the following treatments:

(1) no cut, (2) one cutting on July 29, (3) one cutting on August 21, or (4) one cutting on July 29 and one on August 21. All cutting treatments were at a height of 7.5 cm. The non-hardy cultivar consistently produced more nitrogen per hectare in the top growth over all treatments. Also, there was more nitrogen per unit area produced by the roots in the non-hardy cultivar in all treatments except the July 29 cutting in which the two cultivars produced nearly equal amounts, differing by only 0.1 kg/ha.

Differences in the amount of nitrogen fixed have also been reported by Sharma et al. (1973). Based on plant material analysis, they reported that a quick-growing Indian cultivar fixed more nitrogen than an Australian cultivar.

Physiological Effects on Nitrogen Fixation

Of many factors which could be considered in this section (e.g., respiration, photorespiration etc.), only two will be discussed.

Plant growth stage

Halliday and Pate (1976) indicated that nitrogen fixation was detectable in white clover plants 2 weeks after germination. Fixation continued to increase up to 9 weeks at the termination of the greenhouse experiment. Also analyzed were top and root growth sections (20 x 20 cm by 10 cm deep) from

a white clover-ryegrass pasture. In the first year of growth, there was no acetylene reduction activity during the November through February period when the average soil temperatures were only 4 to 5°C. Once growth began in March there was a rapid increase in acetylene production which continued to increase until the pasture was harvested in late May. It is interesting to note that the authors report finding some pink nodules on the roots in the months during which no acetylene reduction activity was detectable. No explanation was given for the apparent anomaly of pink color and inactive nodules.

Masterson and Murphy (1976) studied acetylene reduction rates in white clover at six locations. At all locations, the rate of acetylene reduction declined after flowering even though the decreases were not at the same time interval after the plants flowered at all locations. At one location, acetylene reduction rates decreased at flowering and increased after flowering was completed. At this time, the rate once again declined to a rate as low as it had been 3 months prior to flowering. It was reported that at all sites acetylene reduction activity began 3 weeks earlier with dry soils as compared to wet. Temperature of the dry soils was lower than the wet soil indicating that wetness may inhibit nitrogen fixation rates more than cool temperatures. The mean soil temperature (25 mm depth) for the 6 days prior to and ending on sampling day was highly correlated with acetylene reduction activity. This research clearly shows that the rate of

acetylene reduction cannot be attributed solely to stage of growth but rather that it is interrelated with several other environmental factors.

Pate (1958) used Kjeldahl analysis of the nodules to assess the rate of nitrogen fixation in vetch (Vicia sativa L.). It was reported that the percentage nitrogen of red nodules increased with age of the plant with a maximum being reached at early flowering. Number of nodules and weight per nodule decreased at the onset of flowering. Those nodules growing on the main roots were found to have a consistently higher percentage nitrogen than those growing on branch roots.

Three tickclover (Desmodium spp.) species were used to determine the effect of flowering on nodulation in tropical pasture legumes (Whiteman, 1970b). Desmodium sandwicense E. Mey has an indeterminate flowering habit, and plants flower throughout the entire season. Spanish tickclover (Desmodium uncinatum (Jacq.) DC) begins flowering in April and greenleaf desmodium flowers in late May to early June. Plants were first grown in a greenhouse, then transplanted to the field. To obtain the nodules, a 929 cm² area 20.3-cm deep centered over a plant was taken. In greenleaf desmodium, maximum nodulation occurred 3 months before flowering but, in the earlier flowering Spanish tickclover, maximum nodulation was 1 month before flowering. Nodulation in D. sandwicense was similar to the other two species in that nodule dry weight per plant increased up to first flowering after which the rate of

increase lessened up to second flowering. In all three species the number of nodules reached a maximum before a rapid declining phase.

Peas were grown in a greenhouse with a 16-hr photoperiod at 15°C to determine the effects of flowering and fruit formation on the supply of photosynthates to nodules (Lawrie and Wheeler, 1974). The life cycle of these plants is only 6 weeks. Acetylene reduction was at a maximum in 3-week-old vegetative plants and nodule mass was highest between 3 and 4 weeks. When plants were allowed to photosynthesize for 30 min in $^{14}\text{CO}_2$ it was determined that the rate of photosynthesis doubled between 3 and 4 weeks, whereas, amount of labeled photosynthates in nodules declined by 60% during this time period from flowering to fruiting. If flowers were removed as they formed during a 2-week period, the amount of labeled photosynthates and nitrogenase activity in the nodules was significantly increased over that of control plants.

In soybeans, the rate of acetylene reduction has been shown to increase during the period of flower initiation to midbloom (Wahua and Miller, 1978) or shortly after flowering (Lawn and Brun, 1974) depending on cultivar. Rate of acetylene reduction remained constant from midbloom to midpod-filling stage (Wahua and Miller, 1978).

Photosynthesis

There is little information in the literature concerning effects of photosynthate supply on nitrogen fixation in alfalfa. Much of the research concerning this has been done with soybeans. Comparable research is needed on alfalfa to determine if the results obtained with soybeans and other annual legumes is applicable to a perennial forage species such as alfalfa.

It is known that the carbohydrate supply in alfalfa roots reaches a maximum at full bloom (Graber et al., 1927) while simultaneously nodule senescence occurs (Vance et al., 1980). One might conclude from these observations that nodules are noncompetitive sinks for carbohydrates produced during photosynthesis.

Sheehy et al. (1979) used acetylene reduction rates and net carbon exchange (NCE) rates to determine the relationship between nitrogen fixation and photosynthesis in 'Vernal' alfalfa. From an initial population, eight clones were selected based on leaf NCE. The clones selected were grouped for high, medium, or low rates of NCE. Whole plant NCE and acetylene reduction rates were then determined on these eight clones. Plants with high NCE rates also had high rates of acetylene reduction. The workers concluded that "these results indicate that plants having high photosynthesis tend to have high nitrogen fixation."

Subterranean clover was grown under controlled environ-

mental conditions to study the effects of diurnal variation in light and temperature on acetylene reduction rates (Eckart and Raguse, 1980). Four treatment combinations were used in which both light and temperature were held constant, both were diurnally varied, or one was diurnally varied and the other held constant. There was a diurnal variation in acetylene reduction rate when the temperature was diurnally varied, regardless if a constant or diurnally varied light source was imposed on the plants. Their results indicated that subterranean clover was more dependent on temperature variation than on light. Apparently, the " N_2 fixation by root nodules of this species is buffered against short-term changes in photosynthate supply." These results were obtained when the plants were in the sixth trifoliate leaf stage, a period when active nitrogen fixation should be occurring.

Lawn and Brun (1974) studied nitrogen fixation in two soybean cultivars, 'Clay' and 'Chippewa 64'. At the end of the flowering period, one of the following treatments was imposed: (1) supplemental light, (2) 25% shade, (3) removal of 50% of pods, (4) 60% defoliation. Supplemental light and depodding increased the source:sink ratio and caused an increase in nodule activity, as compared to the control. On the other hand, shading and defoliation decreased the source:sink ratio, causing a decrease in acetylene reduction rate below that of control plants. The increase in total nodule activity resulted from an increase in specific nodule

activity, nodule number, and nodule fresh weight per plant. Decreases in total activity after flowering were associated with a decrease in specific nodule activity as indicated by green nodules.

In research of Chi-Ying et al. (1975a), soybean plants were girdled to stop the flow of assimilates to the roots. Girdled plants had reduced acetylene reduction rates within a few hours. The researchers suggested that this implied low root reserves. They also reported that at low water potentials the rate of acetylene reduction was correlated with that of photosynthesis, i.e., if photosynthesis is inhibited so is acetylene reduction.

In a review article, Hardy and Havelka (1975) reported that the supply of photosynthates affects nitrogen fixation in field-grown legumes. They cite several articles which support the supposition that any factor which decreases the supply of assimilates (e.g., decreased light intensity, defoliation) will cause a similar decrease in nitrogen fixation. Removal of pods and carbon dioxide enrichment were also discussed in this article as beneficial for nitrogen fixation.

Abu-Shakra and Habib (1979) have shown that in a delayed leaf-senescing soybean line, nitrogenase activity in the roots was maintained during seed maturation while enzyme activity of 'Corsoy', a normal-senescing line, declined during this same time period. Nitrogenase activity was maintained without

a loss of seed yield.

In a greenhouse study, peas were grown with a 16-hr photoperiod at 15°C for the 6-week life cycle of the plant (Lawrie and Wheeler, 1973). After placing 3-week-old plants in darkness for 62 hr, there was no detectable acetylene reduction after returning to light. Increasing of the time period during which the plants were subjected to darkness increased the time needed for the plants to reach a rate of acetylene reduction equal to that of controls. It was found that the accumulation of radioactive-labeled assimilates was much reduced after darkening and returning to light. There was a close relationship between acetylene reduction and accumulation of recently produced assimilates. There was little difference between radioactivity of nodules in plants growing in the light versus those in the dark, indicating that assimilates were not stored in nodules but rather metabolized immediately.

Environmental Effects on Nitrogen Fixation

There are a host of environmental factors that can affect the rate of nitrogen fixation. Many of these factors are interwoven in their effects. Any of these environmental factors can cause nodulation stress which is:

...defined as that stress which adversely affects the formation and function of nodules such that there is a greater difference in nitrogen assimilation between

inoculated plants and plants supplied combined nitrogen under stress conditions than there is under optimal conditions (Gibson, 1976).

Temperature, moisture supply, and light are such important factors that they will be discussed separately insofar as possible.

Temperature

Day and Dart (1969a) measured nodulation and nitrogen fixation in nine legumes which included alfalfa. They measured acetylene reduction over a temperature range of 3 to 45°C. Nitrogenase activity was affected most at the temperature extremes with little differences occurring in the range of 10 to 30°C. A temperature of 45°C caused an irreversible loss of acetylene reduction activity. As much ethylene was produced in 48 hr at 3°C as at 20°C in 8 hr. Ethylene production ceased to form at the end of each of these time periods.

In a review article reported by Gibson (1971), it was pointed out that temperature was considered to be of utmost importance for the nitrogen fixation process to occur. The process is inhibited by a high root temperature, but the plant host and strain of bacteria interact to give varying degrees of inhibition. There is no effect of high shoot temperature when the root temperature ranges from 20 to 30°C.

The effect of four temperatures (24 (control), 32, 36, and 40°C) on alfalfa grown in a greenhouse was reported by Munns et al. (1977). Pots containing the plants were heated

for 4 hr each day for four consecutive days. Acetylene reduction was measured on days 1, 2, and 5 of the treatment period. Treatments were applied to 4-week-old plants 9 days after clipping. Acetylene reduction decreased to 41, 1, and less than 1% of the control at day 5 for the 32, 36, and 40°C treatments, respectively. It took only 1 hr for the decrease to occur at 40°C but 4 hr at 36°C. It was reported that the surface 5 cm of soil in the field contained less than 10% of the total nodule number where the temperature was above 30°C. Because of this, the authors thought that a high soil temperature was unlikely to inhibit nitrogen fixation in the field unless nodulation was confined to shallow depths. This is in contrast to other work with alfalfa which reports over 80% of nitrogen fixation activity occurring in the surface 7.5 cm and nearly 60% in the surface 2.5 cm (Sinclair et al., 1976).

Total nitrogen percentage was measured in subterranean clover grown on agar in a growth chamber (Gibson, 1969). He used various combinations of root and shoot temperatures for varying lengths of time under dark or light conditions. Three strains of bacteria were used. Results indicated many interactions depending on root temperature, shoot temperature, illumination, and strain of bacteria. In general, however, those strains of bacteria which were known to be sensitive to high temperature showed a decrease in nitrogen fixation as daylength varied: 4-, 8-, 12-, or 20-hr days, and continuous

illumination, at 30°C.

In another greenhouse study, alfalfa was grown in sand until full bloom, clipped to 5 cm, after which the pots were subjected to either 16 or 30°C temperatures in water baths (Barta, 1978). Acetylene reduction values were obtained every 2 weeks on four pots. A second clipping was taken 4 weeks after the first one. Plants subjected to the 16°C temperature treatment had significantly higher acetylene reduction rates at either the vegetative or flowering stage as compared to those subjected to 30°C. The researcher suggested that some nodule degradation may be occurring at 30°C, such that the exact effect of temperature was questionable.

Temperature was reported to have the greatest effect on growth of nodules in cowpea (Vigna sinensis Endl. Ex Hassk.) but it was also influenced by light intensity and nitrate in the soil (Dart and Mercer, 1965). The authors postulated that extreme temperatures (higher than 30°C) may decrease Rhizobium growth rate so there are insufficient numbers to infect the plant until secondary root growth occurs causing more nodules on these roots.

Rogers, cited by Gibson (1976), reported that alfalfa made better regrowth when supplied with combined nitrogen under high temperatures than those without nitrogen. Both treatments were nodulated. When returned to a favorable temperature regime, nodulated plants without combined nitrogen made comparable growth to those with nitrogen provided after

a second cutting. This indicated that the nitrogen fixation system had recovered from the adverse effects of high temperatures. Gibson cites other researchers who have found nodulation and nitrogen fixation to be dependent on temperature in subterranean clover, lupine (Lupinus angustifolius L.), soybeans, and white clover. It is pointed out that "species differences, plant age, and other features of the environmental conditions under which the plants are grown, influence the nature of the results obtained." Gibson (1976) further stated that in some species the amount of nodule tissue produced may vary depending on the temperature, but because of a higher specific activity a lower nodule mass may not necessarily mean a lower rate of nitrogen fixation.

Moisture

Reports of research directly concerning moisture supply and its effect on nitrogen fixation in alfalfa are very limited. One report (Nutman, 1976) stands out and mentions water supply as it affects nitrogen fixation in alfalfa. In the experiment reported, the effects of fertilizers were being evaluated. These effects varied at different locations. Climatic factors, including moisture supply, were thought to play a significant role in the amount of nitrogen fixed at each location.

Engin and Sprent (1973) grew white clover in a controlled environment chamber and at 4 weeks of age applied four water-

supply treatments (optimum (100%), 75, 50, and 0% of optimum) to determine the effect on nitrogen fixation. Relative humidity was 75% for the 16-hr light period and 65% in the dark for 8 hr with a 21°C/19°C temperature for the same time periods, respectively. Acetylene reduction of whole plants during a 24-day period indicated that water stress caused nodule activity to be less than 100% of optimum. This effect became more apparent as the time of stress increased. Stress caused little change in nodule fresh weight. Nodule activity decreased more rapidly under more severe stress conditions. In the 0% treatment, nodule activity was below that of day 1 after 12 days of the treatment while it took 19 days for acetylene reduction to fall below that of day 1 under 50% optimum conditions. The observed decrease in acetylene reduction resulted from a decrease in efficiency. For example, in the 75% treatment, nodule weight decreased 20% but ethylene production per unit fresh weight per hour decreased more than 50%.

The type of nodules on the plant influences the effect of water stress on nitrogen fixation (Sprent, 1976). Broad bean (Vicia faba L.), which has meristematic nodules, recovers from water stress by regrowth of existing nodule tissue in 2 to 3 days after watering while soybeans, having round nodules, first sheds them, and then grows new nodules after watering. The growth of completely new nodules takes longer than regrowth of nodules from existing tissue. Regrowth has been shown to

occur in alfalfa nodules, which were partially senescent, from a meristematic zone (Vance et al., 1979). Sprent (1976) also quoted reports that in field beans and soybeans, water-logging can cause plants to have fewer nodules per plant, smaller size, higher water content, and less acetylene reduction activity than plants under optimal water conditions.

Chi-Ying et al. (1975b), working with soybeans, concluded that acetylene reduction was not detectable at either a water potential in the soil of -19.5 bars or in a flooded soil. In between, there was a sharp optimum occurring at approximately -1 bar. By removing wet soil from the roots they were able to show a 1.6- to 1.7-fold increase in acetylene reduction over plants without the soil removed, suggesting that nodule activity was reduced in wet soil because of impaired gas exchange through the available soil pore space.

Light and shading

It is difficult to separate light and shading effects without discussing supply of photosynthates. There are some pertinent articles, however, which report the effect of light, or shading, or both on the rate of nitrogen fixation without discussing a known rate of photosynthesis. Whether the results reported resulted from changes in the photosynthate supply or to a change in some other factor such as nitrogenase activity cannot be ascertained, unless the researcher had specifically assayed for these compounds.

Day and Dart (1969b) grew seven legumes under varying light conditions ranging from 15,000 to 30,000 lux. Included were alfalfa, subterranean clover, red clover, and hairy vetch (Vicia hirsuta (L.) S.F. Gray). Nitrogen fixation activity was assessed by the acetylene reduction method. This range of light intensities had little effect on nodule number on the primary roots of alfalfa and red clover, whereas in subterranean clover and hairy vetch, the number of nodules increased with an increase in light intensity. In all species examined, an increase in light intensity caused an increase in growth, nitrogen fixation, and nodule weight.

Birdsfoot trefoil (cultivar 'Tana') and 'Vernal' alfalfa were grown under varying levels of shade to assess the nodulation response (Cooper, 1966). The four treatments applied were: (1) full sunlight, (2) 51% shade, (3) 76% shade, and (4) 92% shade. A saran shade cloth was used to achieve these shading levels. Shade produced is about the same type of shade as found under trees up to 75% shade. Above 75% shade under a saran cloth, however, there is less blue light absorbed by the saran cloth than by the tree leaves. This results in a different quality of light. The average number of nodules per plant at a 15.25-cm depth was 6.4, 7.9, 8.2, and 7.0 at full sun, 51, 76, and 92% shade, respectively, for alfalfa and 2.2, 2.7, 3.1, and 0.6 for birdsfoot trefoil, respectively.

In a review article, Gibson (1976) cites reports where nodulation in soybeans and nitrogen fixation in sub-

terranean clover is affected by daylength. Also cited were reports on the response of subterranean clover to varying light intensity. Specifically, he cited an experiment where subterranean clover was grown at either a low or high light intensity for 20 days, after which some plants from each light regime were transferred to the other light intensity. Those transferred from the low to high light intensity showed a 50% increase in acetylene reduction activity in 5 hours, as compared to those plants which were not transferred. Plants transferred from the high to low light intensity showed a 40% decrease in acetylene reduction activity within 5 hours, on a per plant basis.

Ten-week-old white clover plants grown in a greenhouse were subjected to 15% of normal daylight (Chu and Robertson, 1974). They showed a reduction in number and weight of nodules per plant but not in acetylene reduction rate although a slight greening of nodules did occur. The authors stated that "shading reduced the ratio of nodule weight to root weight, indicating that nodule growth was reduced to a greater extent than root growth." Shading caused the same effects as defoliation although effects were less severe and slower to appear.

Butler et al. (1959) grew white clover, red clover, and trefoil (Lotus uliginosus) in glass front boxes in a greenhouse. They examined the effects of shading and defoliation on nodulation. Shading was 75% of full sun and clipping

treatments were to 1.25 cm every 11, 12, and 14 days for white clover, red clover, and trefoil, respectively. Defoliation caused a drastic reduction in nodules per plant in red clover and trefoil but nodule number actually increased nearly 50% in white clover as compared to untreated control plants. In all three species, shading reduced the number of nodules significantly. Trefoil had no pink nodules from either treatment. Also, trefoil was more adversely affected than the other two species tested due to the treatments imposed.

Wahua and Miller (1978) used acetylene reduction to determine the effects of six levels of shade on field-grown soybeans. Under 20, 47, and 63% shade, acetylene reduction activity was highest at midbloom, after which there were decreases. Acetylene reduction activity under 80% shade was unaffected by growth stage but was lower than plants under 20 or 47% shade. A shade level of 93% caused all acetylene reduction activity to cease. Of interest was the fact that plants growing with 20% shade had a higher acetylene reduction activity than those grown under full sun. Apparently there is sufficient light at 20% shade to saturate the canopy. Specific nodule activity decreased linearly with increasing levels of shade.

In another experiment with soybeans, the greatest amount of acetylene reduction per plant was reported for plants grown under 18% shade (Trang and Giddens, 1980). These results were

obtained when the greenhouse-grown plants were 37 days old. No explanation was suggested for the highest activity occurring at less than full sun. It was reported that there were fewer nodules per plant as the percentage of shade increased. Yet, acetylene reduction activity per unit weight of nodule increased with an increase in shade up to 62%, this being the highest shade level in the study.

Soil factors

A host of soil factors affect nodulation and nitrogen fixation rate of legumes. Only some of the factors will be mentioned, particularly as they relate to nodulation and nitrogen fixation.

Moustafa et al. (1969) applied nitrogen at 89.7 kg/ha to white clover 4 months after seeding. They analyzed for nitrogen fixation by using acetylene reduction. They reported that plants fertilized with nitrogen had acetylene reduction rates 23 to 30% lower than that of plants not receiving commercial fertilizer nitrogen.

Combined nitrogen may inhibit root hair infection, nodule initiation and development, or nodule function depending on the plant species, strain of bacteria, level of applied nitrogen fertilizer, and placement within the soil (Gibson, 1976).

It has been shown that nodulation and nitrogen fixation rates can be increased by use of potassium fertilizer at rates of 673 kg/ha of potassium as K_2SO_4 (Collins et al., 1979).

Bell and Nutman (1971) have also shown an enhancement in nitrogen fixation by adding potassium and phosphorus.

Other soil factors that may influence nitrogen fixation are the levels of calcium, manganese, and aluminum in the soil (Keyser and Munns, 1979). Low pH and aluminum toxicity have been shown to have an inhibitory effect on Rhizobium causing a decrease in viability and slower growth rate.

A soil pH of less than 6.0 was associated with a decrease in nitrogen fixation rate in alfalfa (Rice et al., 1977). The cause of this lowered rate of nitrogen fixation was attributed to low Rhizobium numbers and a decreased rate of nodule initiation.

CHAPTER III. DINITROGEN (C_2H_2) FIXATION IN ALFALFA
(Medicago sativa L.) AS AFFECTED BY PLANT
DENSITY, CUTTING MANAGEMENT, AND CULTIVAR

Introduction

Alfalfa (Medicago sativa L.) was recognized many years ago as an important source of nitrogen (Lyon and Bizzell, 1934). Recommended current practices, which are geared to maximizing yield and quality, may or may not result in the maximum rate of nitrogen fixation. It is not known which cultural factors such as cultivar, plant density, and cutting management will result in maximum nitrogen fixation rates in alfalfa (Vance, 1978).

Cutting has been shown to cause a reduction of nitrogen fixation in white clover (Trifolium repens L.) (Moustafa et al., 1969) and alfalfa (Vance et al., 1979). Nodules on alfalfa have been shown to senesce within 4 days after clipping at the 1% flower stage (Vance et al., 1980). Moustafa et al. (1969) reported that complete defoliation of white clover caused a significant decrease in acetylene reduction rate. The lowest rate was detected on the sixth day after cutting, after which it began to increase on the eighth day and reached the same level as that of control plants by 34 days after cutting. Langille and Calder (1971) reported more nodulation on birdsfoot trefoil (Lotus corniculatus L.) with a decreased frequency of harvests.

Reports of the effect of plant density on the rate of

nitrogen fixation in forage legumes are limited. Phillips and Bennett (1978) reported that in subterranean clover (Trifolium subterraneum L.) there was an increase in the rate of nitrogen being fixed per hectare as the plant population increased. They studied pure clover stands and reported that peak acetylene reduction activity occurred later in the season as the plant density decreased. In soybeans (Glycine max (L.) Merr.), the rate of acetylene reduction per plant has been reported to increase with a decrease in plant population (Hardy et al., 1972). This same effect occurs in broad beans (Vicia faba L.) (Sprent and Bradford, 1977).

A nonhardy cultivar of alfalfa consistently produced more nitrogen per hectare in the top growth than a hardy cultivar (Stickler and Johnson, 1957). Duhigg et al. (1978) reported that in the semidormant cultivar of alfalfa, 'Mesilla', plants selected for high acetylene reduction activity had a larger number of nodules with darker red color, larger root system, more top dry weight, and more total nitrogen in the top growth than the original cultivar from which they were selected. Differences in nitrogen fixation rates resulting from cultivar differences have also been shown to occur in subterranean clover (Nutman, 1961) and white clover (Connolly and O'Keeffe, 1979).

The objective of this study was to determine how plant density, cutting management, and cultivar affects the rate of nitrogen fixation in alfalfa.

Materials and Methods

Experimental design, location, and treatments

The experiment was located at the Iowa State University Agronomy and Agricultural Engineering Research Center 13 km west of Ames. Soil type at the site is a Webster silty clay loam, classified as a fine loamy, mixed mesic typic Haplaquoll. The site had been previously cropped to soybeans in 1975 and 1977, to grain sorghum in 1976, and summer fallowed in the summer of 1978. Alfalfa was seeded on August 9, 1978.

Each plot, consisting of 13 rows spaced at 20.3 cm, was 2.44 x 6.40 m in size and was separated on all sides by .91-m alleyways. Three treatment variables were used in this study making a total of 18 treatment combinations. Treatments were: (1) cultivar, 2; (2) seeding rate, 3; and (3) cutting management, 3. Cultivars selected were 'Agate', an alfalfa noted for its fall dormancy and rated as winterhardy (Barnes and Frosheiser, 1973) and 'WL 318', moderately fall dormant and thus moderately winterhardy (Beard and Kawaguchi, 1978). To insure adequate infection, seed was inoculated with a commercial strain of Rhizobium just prior to seeding. Seedlings were made in a north-south direction with a single-row Planet Jr. planter. Seeding rates were 6.7 (low), 13.5 (medium), and 26.9 (high) kg/ha. The three cutting managements imposed were a no-cut management system, a three-cut management system, and a four-cut management system. The 18 treatment combina-

tions were arranged in a randomized complete block design with three replications.

All plots received 186 kg/ha of K_2O in the fall of 1978 prior to seeding. In 1979, 320 kg/ha of K_2O was applied in a split application. The corresponding amount in 1980 was 300 kg/ha. Fertilizer was applied in early spring and again after the first harvest of the three-cut management system. All plots were fertilized at this time. In addition, in 1979, 90 kg/ha of P_2O_5 was applied on June 5.

To control insects, primarily leaf hoppers, all plots were sprayed with malathion as needed.

Acetylene reduction

Field sampling procedure The second, third, and fourth rows from the east side of each plot and the sixth, seventh, and eighth rows were sampled in 1979 and 1980, respectively, for the measuring of potential nitrogen fixation by use of the acetylene reduction method (Hardy et al., 1968; Sinclair, 1973). A 7.3-cm diameter x 10.2-cm soil core was taken. Each core contained at least one plant. Plant tops were clipped at ground level before sampling. As sampling progressed down the row, if the last plant sampled was not separated from the next by at least 15 cm, then a single plant or group of plants was left and the next plant or plants were taken for that sample. On each sampling date, all plots were sampled three times, once from each row as described previously.

Roots longer than the core were clipped to the depth of the core. Thus, nitrogen fixation activity was measured to a depth of 10.2 cm.

For the first of three samplings on any sampling date, soil cores containing plants were immediately transferred to wide-mouth plastic bottles, 2 liters in size. The plastic bottles had a screw-on lid and the bottle was fitted with a rubber septum to allow for transfer of gases. For the second and third repetition on any sampling date, samples were first placed in plastic bags just large enough to enclose the core. The cores were then placed in the plastic bottles after transporting them to the laboratory. This procedure was followed because only one set of the large bottles was available. Thus, while one set of samples was being analyzed, the next sampling could be made allowing for the three samplings to be completed in approximately 10 hours. All samples were taken between 7:00 a.m. and 3:00 p.m.

Laboratory procedure Bottles containing the soil cores with alfalfa roots were filled with 200 cc of acetylene after removing an equal volume of air, thus a 10% (v:v) mixture of acetylene with air resulted. After injecting the acetylene, each bottle was inverted once to insure adequate mixing of gases.

Samples were incubated at approximately 22°C for 2 hours before measuring for ethylene production. After incubation, each bottle was inverted once again before withdrawing a 1-ml

sample of gas with a gas syringe. Amount of ethylene produced was measured with a Becker gas chromatograph, model 417, equipped with a 1.3-m column packed with Porapak R. A flame ionization detector was used with a hydrogen flow rate of 30 ml/min and 30 ml/min oxygen. Helium was used as a carrier gas. Injection port and detector temperatures were 130°C with an oven temperature of 70°C. Column pressure was 1 kp/cm². Retention time of ethylene was approximately 45 seconds allowing one sample to be analyzed every 70 seconds. Amounts of ethylene produced were calculated by comparing to a known standard peak of ethylene. All rates are reported as μ moles/hour/core or /plant as noted in the discussion in this paper.

Harvest schedule

Cutting to a height of 7.6 cm was standard. Under the three-cut management system, the first cutting was harvested at approximately 1/10 bloom. Subsequent harvests were equally spaced, with an allowance for the last harvest to occur approximately 4 weeks before the first frost of -2.2°C in the fall. Harvest dates were June 12, July 27, and September 8 in 1979 and June 5, July 23, and September 12 in 1980. The four-cut management system was first harvested at the late-bud stage with the remaining harvests spaced equally throughout the season. The last harvest was taken at the same time as the three-cut management system. Harvest dates were June 5, July 6, August 9, and September 8 in 1979 and May 29, July 1,

August 8, and September 12 in 1980. Plant material remaining on the no-cut management plots after the first year was removed prior to the first acetylene reduction sampling the second year.

Plant density

Assessment of plant density was made 8 weeks after seeding in the fall of 1978 to determine the success of initial establishment. The number of seedlings was counted on four 30.5-cm sections of row which were randomly selected. This count indicated that there was an average of 23.4, 43.0, and 46.4 plants/m of row for the low, medium, and high seeding rates, respectively.

Plant densities following summer harvests were made on October 8, 1979 and September 12, 1980. Two 61-cm sections of adjacent rows located in the southwest corner of each plot were used in each of these determinations. A spade was used to remove the plants to insure accurate counts. The medium plant density was not assessed in 1980 because there were only small significant differences between the medium and high densities the previous year.

In the fall of 1979, there were 19.9, 33.9, and 40.0 plants/m row for the low, medium, and high plant densities, respectively. The low and high density had 11.5 and 17.2 plants/m row, respectively, in the fall of 1980.

For all acetylene reduction samplings, the number of plants contained in each soil core was counted after the production of ethylene had been measured. This value was used to establish if there were differences as a result of plant

densities in the field. The number of plants/core are presented in Table 1.

Results and Discussion

Data will be presented and discussed in two ways:

(1) acetylene reduction rates per soil core (ARPC), (2) acetylene reduction rates per plant (ARPP). All acetylene reduction rate values will be expressed as μ moles ethylene/hour/core or /plant.

The ARPC values can generally be thought of as that amount of ethylene produced per unit area. The reader is cautioned, however, not to extrapolate these values directly to a larger unit area because the rates recorded on a per core basis were always directly over a plant or group of plants. The area between the 20.3-cm spaced rows of alfalfa would likely be typified by lower ARPC values.

Acetylene reduction per core (ARPC)

Mean values of acetylene reduction rates per core (ARPC) are presented in Tables 1 through 3 and in Figures 1 through 6.

Separate analyses of variance were computed on data from each sampling date in each year as well as for the entire year. The analysis of the entire year removed the variability which occurred as a result of sampling date, i.e., those samples taken early in the season were higher than those taken in the later part of the season. The combined-year analysis

Table 1. Mean acetylene reduction rates and mean number of plants per core averaged over all other treatment variables^a

Treatment	<u>Year 1</u>			<u>Year 2</u>		<u>Combined years</u>	<u>Year 1</u>	<u>Year 2</u>
	<u>Apr 30- Oct 1</u>	<u>June 4-Oct 1</u>		<u>Apr 22-Oct 7</u>			June 4- Oct 1	Apr 22- Oct 7
	-----Core-----	Plant		Core	Plant	Core		
-----μ moles ethylene/hour-----							---plants/core---	
Plant density								
Low	1.05	.53	.22a	.24	.12	.65	2.6a	2.0a
Medium	1.01	.53	.14b	- ^b	-	-	4.2b	-
High	.97	.50	.11b	.29	.09	.64	5.3c	3.0b
Cutting management								
No-cut	.91a	.47a	.15	.31a	.13a	.62	3.9	2.4
3-cut	1.08b	.59b	.17	.22b	.08b	.65	4.1	2.6
4-cut	1.06ab	.52ab	.15	.27ab	.10b	.67	4.2	2.5
Cultivar								
Agate	1.06	.57a	.16	.31a	.11	.69a	4.3a	2.6
WL 318	.96	.47b	.15	.23b	.09	.60b	3.8b	2.4

^aNumbers within a column within a treatment followed by different letters are significantly different at the .05 level of probability. Those without letters are not significantly different.

^bData not taken the second year.

Table 2. Means of acetylene reduction rates per core (ARPC) by date in the first sampling year

Date	Plant density			Cutting management ^a			Cultivar	
	Low	Medium	High	No-cut	3-cut	4-cut	Agate	WL 318
-----μ moles ethylene/hour/core-----								
April 30	1.69	1.42	1.67	1.40	1.66	1.72	1.47	1.71
May 9	4.01	3.60	3.92	3.75	3.98	3.81	4.09	3.59
23	4.21	4.23	3.52	4.19	3.56	4.22	3.93	4.05
June 4	2.82	2.60	2.52	2.42	2.72	2.80	2.88	2.41
11	.78	.72	.59	.71	.92	<u>.45</u> **	.81	.58
19	.30	.35	.26	.37	<u>.23</u>	- **	.33	.28
22	.53	.64	.60	.44	-	.75**	.63	.55
28	.41	.40	.45	.29	.55	-	.44	.41
July 5	.27	.27	.31	.21	-	.36**	.30	.27
12	.37	.31	.32	.31	.58	<u>.13</u> **	.36	.31
26	.43	.41	.39	.30	.47	.48*	.43	.40
Aug 2	.28	.30	.21	.33	<u>.19</u>	- **	.32	.20**
8	.48	.65	.56	.54	-	.59	.69	.43**
15	.31	.30	.32	.31	.53	<u>.09</u> **	.32	.30
29	.17	.17	.18	.14	.23	.14*	.17	.17
Sept 7	.17	.19	.20	.12	.23	.20**	.21	.16**
14	.14	.19	.18	.28	<u>.13</u>	<u>.10</u> **	.20	.14**
Oct 1	.18	.24	.19	.22	.25	.14*	.22	.18

^aValues underscored were samplings taken 6 days after a cutting management had been imposed; - indicates data not taken on these dates.

*,**Significant at the 0.05 and 0.01 levels, respectively, among treatments within a factor, within a date.

Table 3. Means of acetylene reduction rates per core (ARPC) by date in the second sampling year

Date	<u>Plant density</u>		<u>Cutting management^a</u>			<u>Cultivar</u>	
	Low	High	No-cut	3-cut	4-cut	Agate	WL 318
-----μ moles ethylene/hour/core-----							
April 22	.32	.47	.07	.38	.72**	.60	.19**
May 2	1.21	1.65*	1.53	1.08	1.68*	1.72	1.14**
15	1.12	1.24	1.74	.91	.88*	1.18	1.17
29	.55	.60	.79	.47	.46*	.64	.52
June 4	.14	.14	.20	.17	<u>.04**</u>	.15	.13
11	.23	.30	.46	<u>.09</u>	<u>.25**</u>	.34	.20**
20	.18	.20	.21	.16	.21	.23	.15**
30	.21	.21	.14	.28	.21*	.24	.18
July 7	.06	.10	.11	.12	<u>.01**</u>	.09	.06
15	.06	.07	.07	.07	<u>.05</u>	.07	.06
22	.06	.05	.04	.05	.08	.07	.05
29	.10	.06	.07	<u>.00</u>	.18*	.08	.09
Aug 7	.07	.06	.07	.04	.08	.07	.05
14	.03	.05	.06	.06	<u>.01*</u>	.05	.03
22	.04	.07	.03	.06	<u>.08</u>	.04	.07
Sept 2	.08	.05	.05	.05	.10	.08	.06
11	.06	.08	.07	.05	.09	.08	.06
18	.04	.04	.11	<u>.01</u>	<u>.00**</u>	.06	.02
Oct 7	.07	.08	.09	.09	.05	.06	.09

^aValues underscored were samplings taken 6 days after a cutting management had been imposed.

*,**Significant at the 0.05 and 0.01 levels, respectively, among treatments within a factor, within a date.

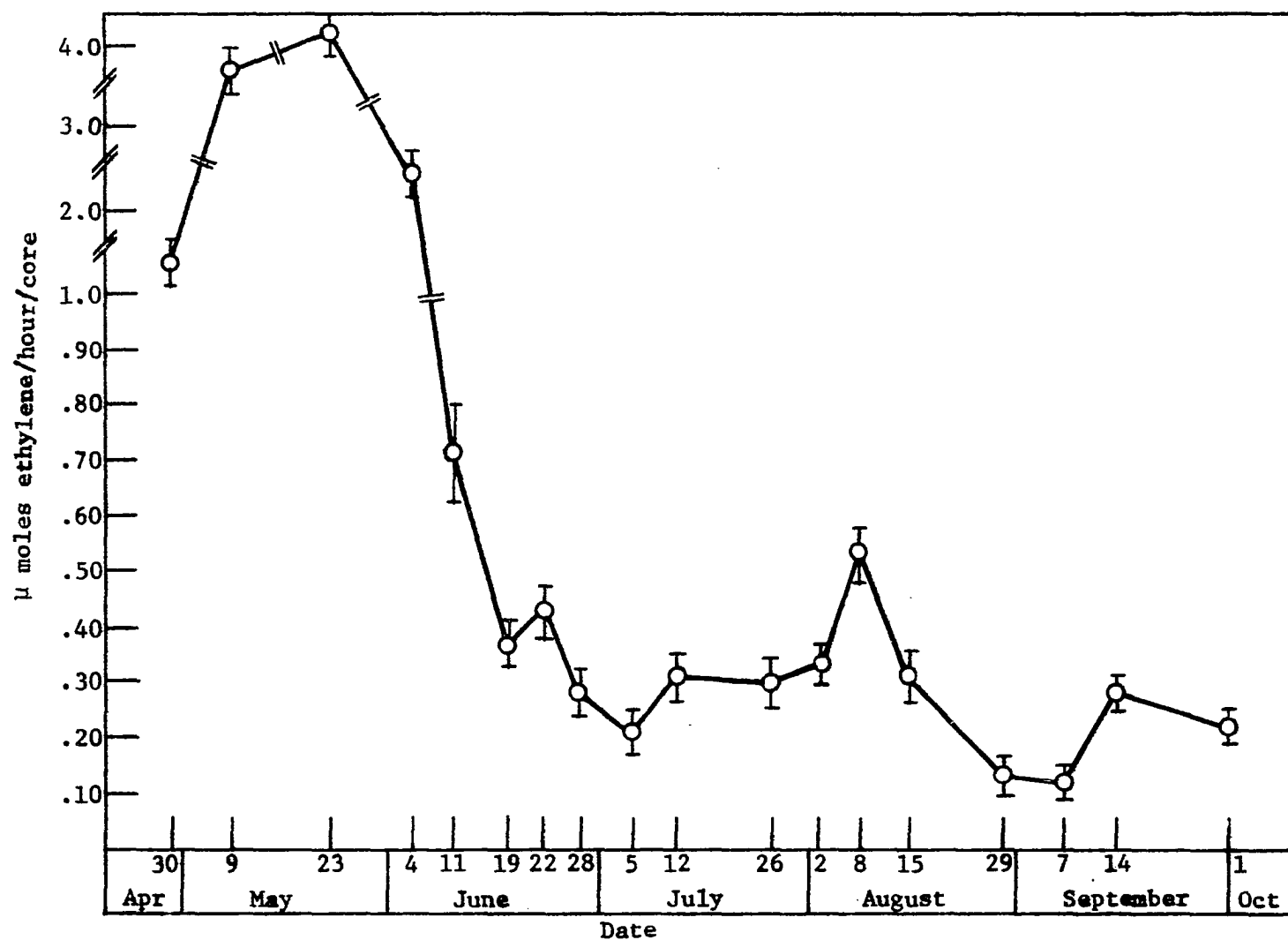


Figure 1. Mean acetylene reduction rates per core (ARPC) in alfalfa of the no-cut management system during the first year; bars are \pm one standard error

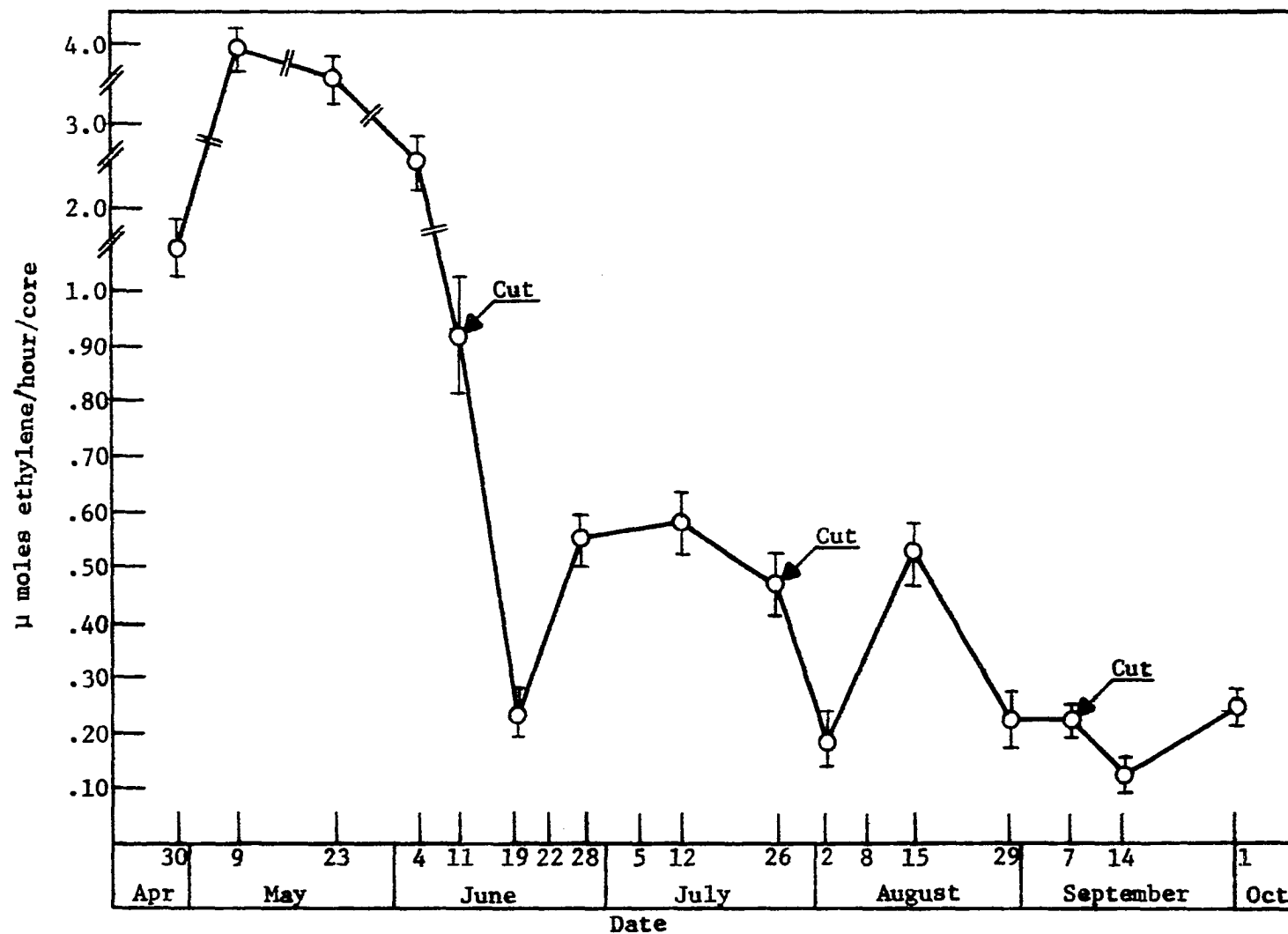


Figure 2. Mean acetylene reduction rates per core (ARPC) in alfalfa of the three-cut management system during the first year; bars are \pm one standard error

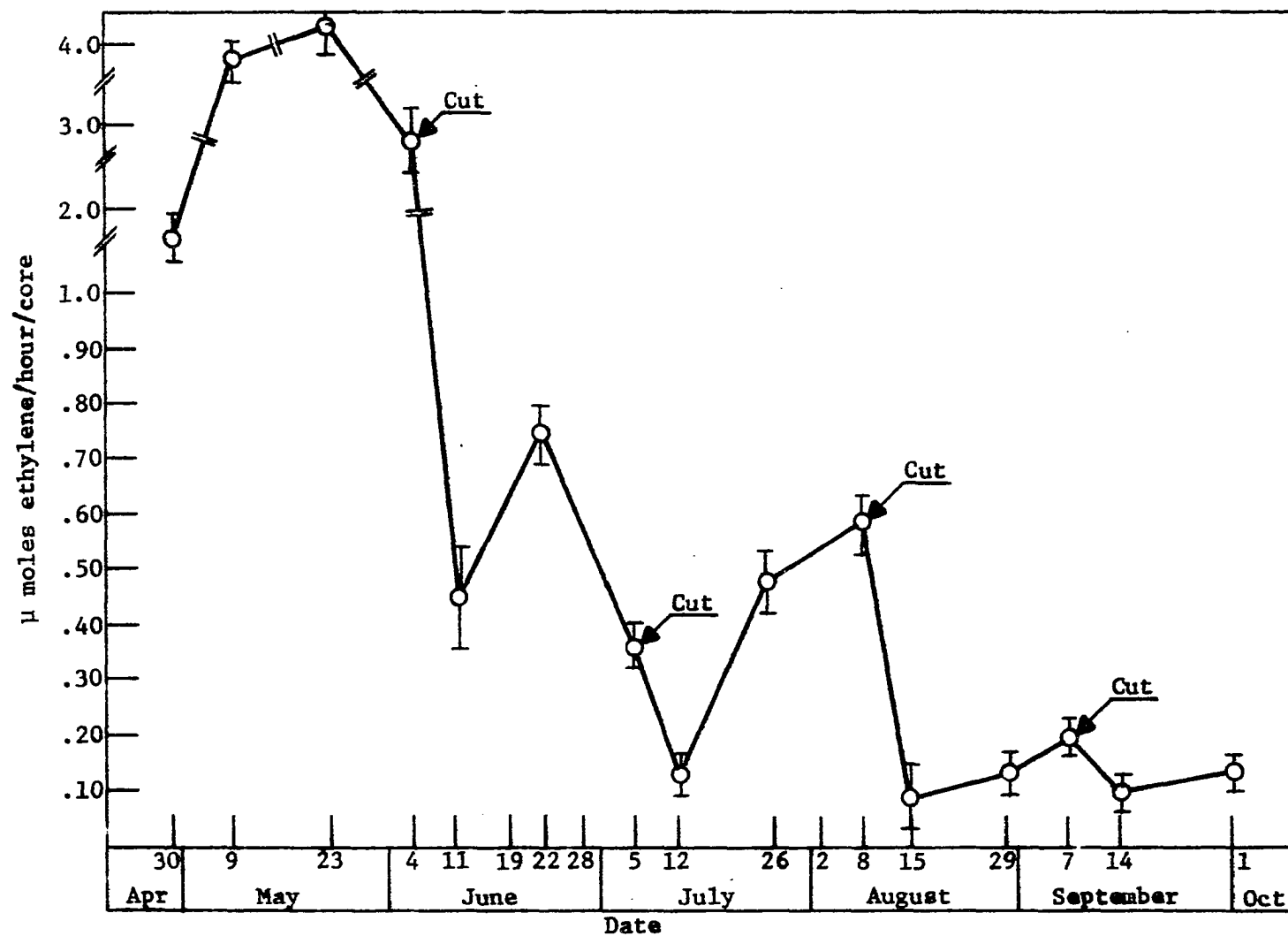


Figure 3. Mean acetylene reduction rates per core (ARPC) in alfalfa of the four-cut management system during the first year; bars are \pm one standard error

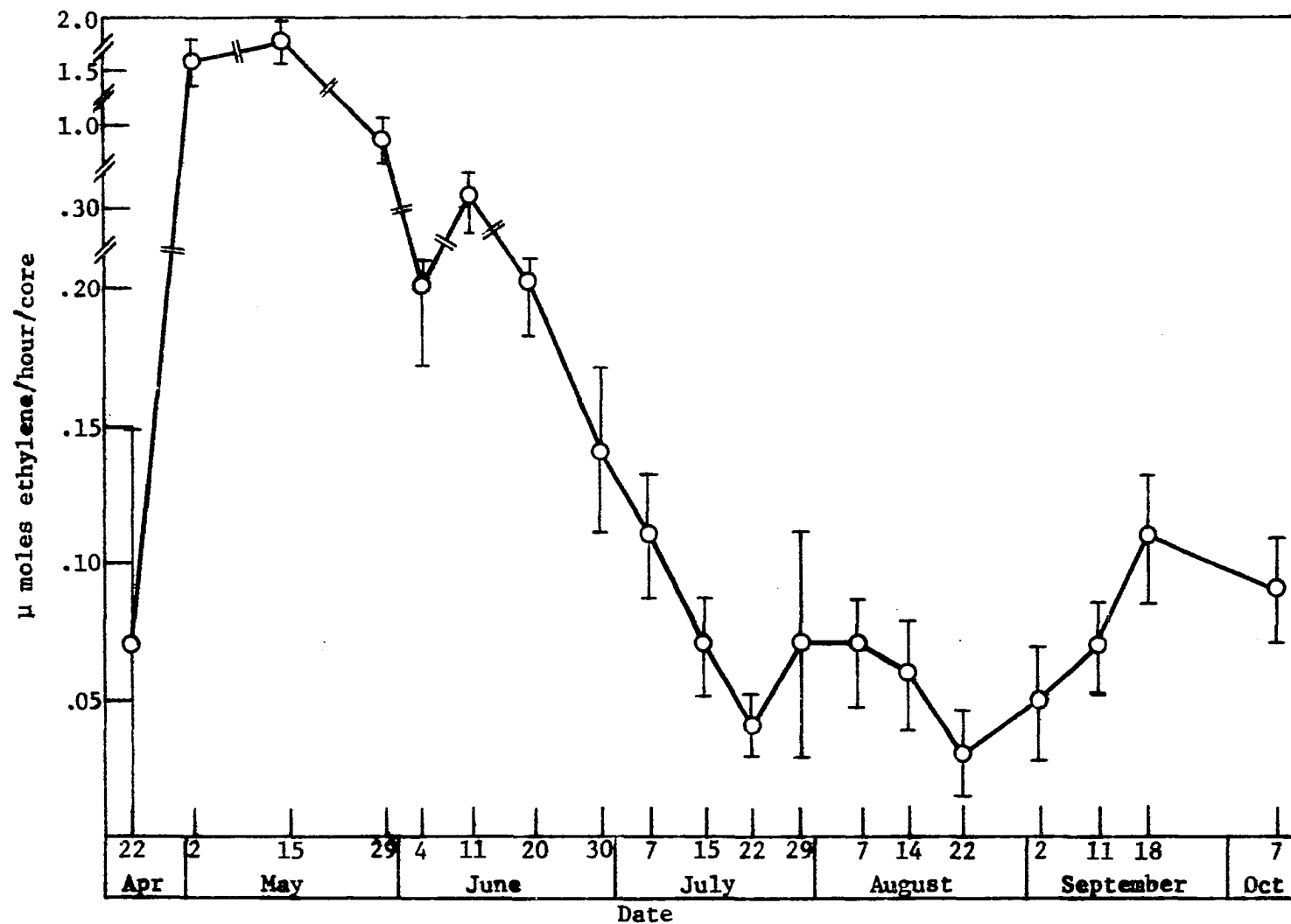


Figure 4. Mean acetylene reduction rates per core (ARPC) in alfalfa of the no-cut management system during the second year; bars are \pm one standard error

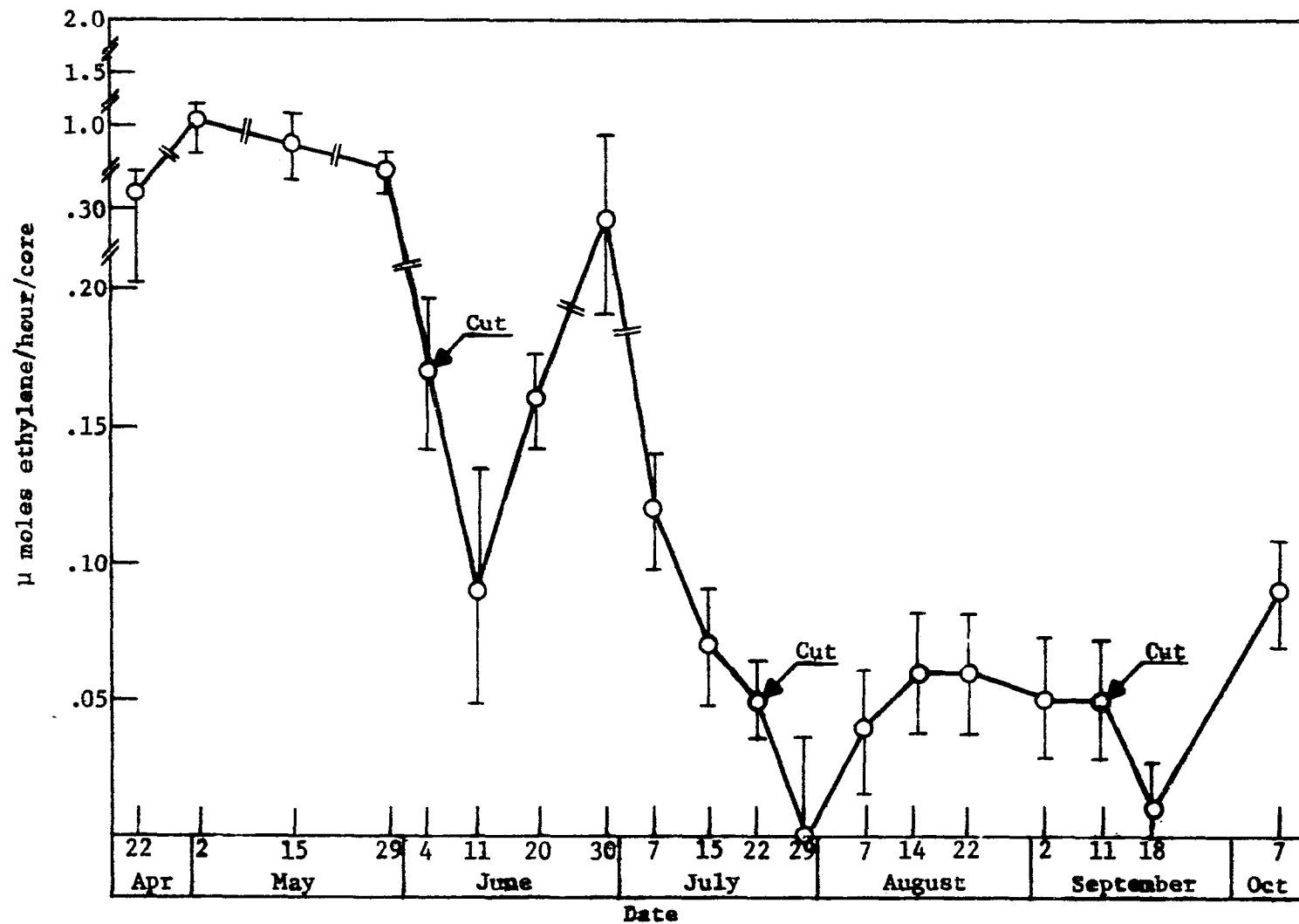


Figure 5. Mean acetylene reduction rates per core (ARPC) in alfalfa of the three-cut management system during the second year; bars are \pm one standard error

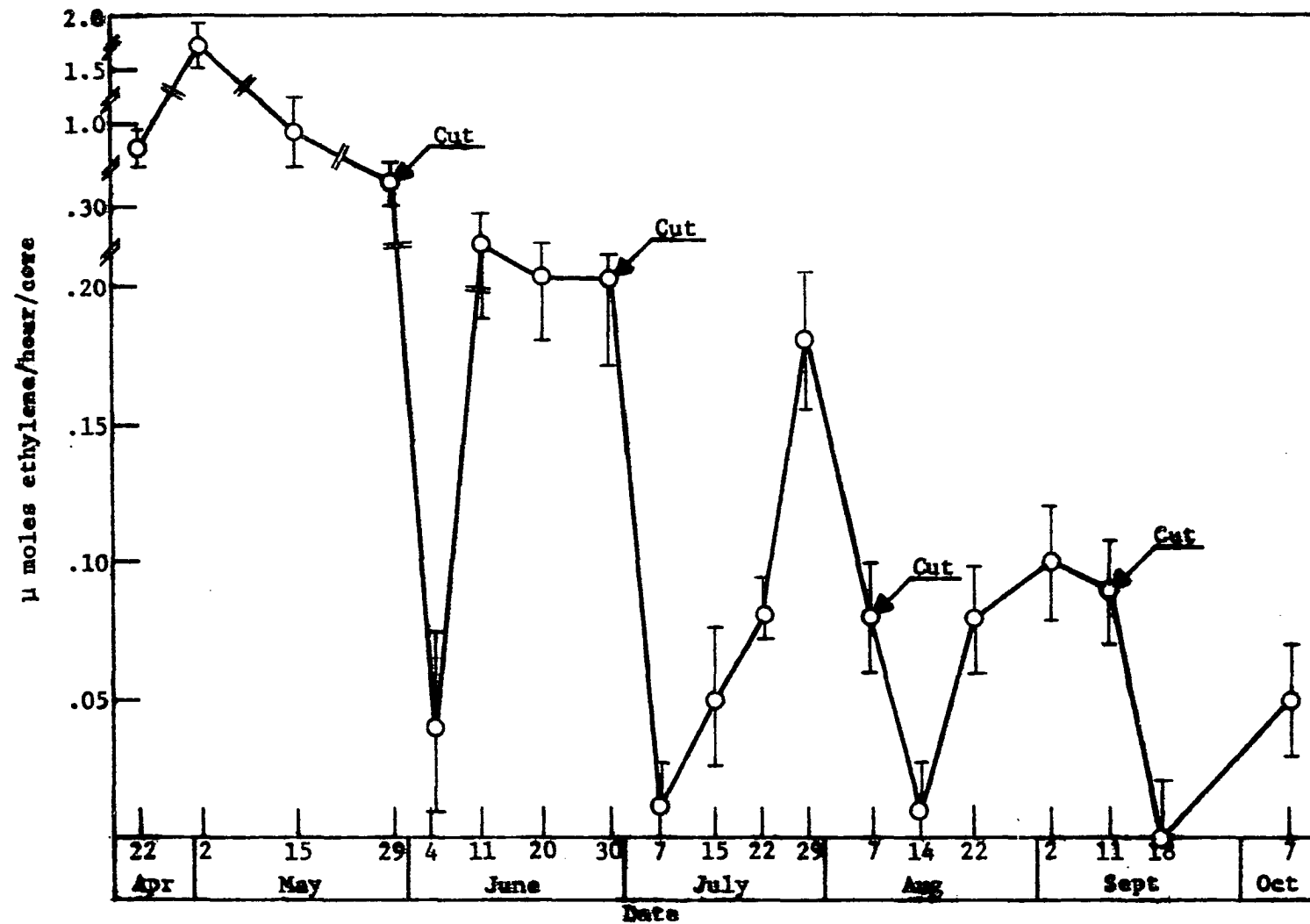


Figure 6. Mean acetylene reduction rates per core (ARPC) in alfalfa of the four-cut management system during the second year; bars are \pm one standard error

removed the effects of sampling date within years and the effects of sampling year on treatments. In the combined-year analysis, it was necessary to delete some of the sampling dates within each year to equalize the number of sampling periods between cutting dates.

Effect of plant density No significant differences resulted in ARPC values at the three plant densities studied in the first year or the two plant densities of the second year, or in the combined-year analysis (Tables 1, 2, and 3). Only on May 2 in the second year did the difference in plant density cause a significant difference in the ARPC values (Table 3). On this sampling date, the high plant density was associated with a higher ARPC value than the low density (1.65 vs. 1.21). Because ARPC values did not differ significantly on other dates as a result of plant density, the difference on May 2 is possibly due to chance. Based on results of this study, ARPC values would not likely be increased even though plant densities are increased.

That plant densities studied were indeed different is shown in Table 1. In both years, there were highly significant ($P < 0.01$) differences in the number of plants/core. The high plant density was originally seeded at a rate fourfold that of the low plant density. Counts of plants/core during the first year, however, resulted in the difference being only twofold greater (5.3 vs. 2.6 plants/core). It is likely that competition among plants at the higher plant density caused

less to survive after emergence.

Effect of cutting management Significant differences
($P < 0.05$) in ARPC values resulting from cutting management were obtained in the individual years (Table 1). No difference, however, was indicated in the combined-year analysis. In the first year, the first significant difference in ARPC values resulting from cutting management occurred on June 11 which was 6 days after the first cutting of the four-cut management system (Table 2). Differences in ARPC values due to cutting management were significant ($P < 0.05$) on all dates following June 11, except on August 8. In the second year, differences in ARPC values were more pronounced for the first nine sampling dates than for the last ten (Table 3). This would suggest that the cutting management imposed the first year was manifesting its effect during the first part of the second year. After June 30 the second year, differences in ARPC values resulting from cutting management were significant ($P < 0.05$) only on those dates in which sampling was done 6 days after one of the cutting managements had been imposed (Table 3). Declines in ARPC values noted after cutting in this study are in agreement with that of Vance et al. (1979) who also worked with alfalfa. Moustafa et al. (1969) reported on similar declines in the species white clover. From Table 1 it appears that cutting the alfalfa causes an increase in the ARPC values measured during the first year. The three-cut management system had significantly ($P < 0.05$) higher ARPC values than the

ARPC values of the no-cut management system. Note, however, in Table 2 and Figures 1, 2, and 3 that 6 days following a cutting, the ARPC values were lower than those for the no-cut management system. Even though the ARPC value initially declined after cutting the alfalfa, it later was greater than the ARPC value of the no-cut management system. Thus, averaged over the entire season the ARPC values for alfalfa cut is higher than when not cut in the first year.

In contrast, the ARPC values tended to be lower as a result of cutting during the second year (Table 1). From Table 3 and Figures 4, 5, and 6, it can be seen that ARPC values were decreased as a result of cutting just as they were during the first year. Note, however, that during the second year, cutting caused the ARPC values to reach zero on July 29 and September 18, whereas this did not happen the first year. Another point can also be noted in data of the second year. The increase in ARPC values above those of the no-cut management system after the initial decrease due to cutting generally was not as great as those during the first year. Thus, when averaged over the entire season, the ARPC values in the no-cut management system during the second year were higher than those in the three-cut or four-cut management system.

There were no differences in ARPC values resulting from cutting management in the combined-year analysis (Table 1). There was a highly significant ($P < 0.01$) cutting management x year interaction, reflecting that the response of ARPC values

to management varied among years. In the first year, the highest ARPC mean value was recorded for the three-cut management system. In the second year, the highest ARPC mean value was noted for the no-cut management system (Table 1). All three cutting management systems had lower ARPC values the second year as compared to the first (Table 1). Note, however, that the decrease in ARPC values from the first to the second year was greater for the three-cut and four-cut management systems compared to the decrease for the no-cut management system. This difference in decline of ARPC values was at least partially responsible for the lack of a significant difference in ARPC values resulting from cutting management in the combined-year analysis.

Effect of cultivar Differences in ARPC values between cultivars were highly significant ($P < 0.01$) the second year and significant ($P < 0.05$) in the combined-year analysis. In the first year, ARPC values differed significantly ($P < 0.05$) as a result of cultivar when the first three sampling dates were not included in the analysis (Table 1). This cultivar effect probably results from differences in the number of plants/core. Agate in both years had more plants/core than WL 318, $P < 0.05$ for the first year and $P < 0.10$ for the second year. Agate averaged 4.3 and 2.6 compared to an average of 3.8 and 2.4 plants/core for WL 318 in the first and second year, respectively (Table 1). The number of plants/core was not recorded in the first year during the first

three sampling periods. These differences in plants/core probably resulted from the differences in winterhardiness and regrowth habit of the two cultivars. Agate is a winterhardy cultivar with slow regrowth, whereas WL 318 is only moderately winterhardy and has faster regrowth. Because of this, WL 318 did not persist as well as Agate during the first winter after establishment or between the two sampling years.

In the first year and in the combined-year analysis, a significant ($P < 0.05$) cutting management x cultivar interaction occurred. A lower ARPC value was recorded for WL 318 than for Agate under the no-cut management system but was equal to Agate under the three-cut and four-cut management systems. These differences cannot be attributed to differences in the number of plants/core. No other explanation is apparent.

In the combined-year analysis, a highly significant ($P < 0.01$) interaction between plant density and cultivar was noted. In this interaction, both cultivars had equal ARPC values for the low plant density. The ARPC value for Agate was higher for the high plant density as compared to the low plant density, whereas, the ARPC value for WL 318 was lower at the high plant density as compared to the low plant density. No explanation can be given for this difference.

Effect of sampling date Stage of growth had an effect on ARPC values, and was indicated by highly significant ($P < 0.01$) differences in each individual-year analysis and in

the combined-year analysis resulting from sampling date. This partially explains the differences associated with cutting management (Figures 1 through 6). The highest ARPC value in each year was reached when the plants were in a late vegetative to early bud stage of growth. The peak ARPC value occurred later in the season in the first year than the second year. A season high of 3.99 was reached on May 23 the first year, while in the second year, the peak value of 1.43 occurred on May 2. Lowest ARPC values for the season were .17 on September 14 the first year and .04 on September 18 the second year. These values are the average of all treatments on a particular sampling date.

Because the cutting schedule each year was on a calendar day basis, the plants on a particular sampling date were in a different stage of growth each year. Plants reached full flower in a shorter period of time in the second year as compared to the first. Thus, during the second year, plants were more often in a flowering stage of growth when sampled than they were during the first year. Because the highest ARPC values were obtained when the plants were in a late vegetative to early bud stage of growth, ARPC values in the second year were significantly lower ($P < 0.01$). The ARPC mean value averaged over all treatments was 1.03 and .29 in the first and second year, respectively. There also was a highly significant ($P < 0.01$) date x year interaction which may be explained by the differences in plant growth stage as discussed

previously. A second possible explanation for lower ARPC values the second year may be a result of nodule distribution. Nodules may have been distributed farther away from the tap root. Thus, these were not included in the core sampled.

In the second year, there was a highly significant ($P < 0.01$) cutting management x date interaction (Table 3 and Figures 4, 5, and 6). On the first sampling date of April 22, the ARPC value of the no-cut management system was considerably lower than the ARPC values of the three-cut or four-cut management system. By May 15, the ARPC value of the no-cut management system was higher than the ARPC value of the other two cutting management systems. The ARPC values of the no-cut management system remained higher than those of the three-cut or four-cut management system until June 20. At this time, the no-cut management system had an ARPC value equal to the four-cut management system and only slightly higher than the ARPC value of the three-cut management system. In addition to this difference in the early part of the season, large fluctuations in ARPC values resulted from cutting the alfalfa in the four-cut management system (Figure 6) which did not occur in the no-cut or three-cut management systems (Figures 4 and 5) after June 20. These differences in ARPC values resulted in the highly significant cutting management x date interaction. A significant ($P < 0.05$) cutting management x date interaction in the combined-year analysis is explained similarly.

Acetylene reduction per plant (ARPP)

Mean values of acetylene reduction rates per plant (ARPP) are presented in Tables 1, 4, and 5.

In the first year, the number of plants/core were not counted on the first three sampling dates. As a result, that ARPP analysis contains fewer observations than for the first-year analysis of ARPC values. An analysis of variance of ARPP values was computed for each sampling year to determine the effects of treatments on the alfalfa within each year. Because of the deletion of the number of plants/core on the first three sampling dates during the first year, an analysis of variance for the combined years could not be computed. An analysis of variance for each sampling date within each year was also computed. This analysis was done to determine the effects of treatments on a particular date.

Effect of plant density During the first year, there was a significant ($P < 0.05$) decrease in ARPP values as plant density increased (Tables 1 and 4). In the second year, differences in ARPP values resulting from plant density were noted to be significant only at the 0.10 level of probability. This inverse relationship between ARPP values and plant density results from competition between plants. It is reasoned that alfalfa plants at higher densities are in greater competition for growth factors such as water, nutrients, and light than at lower densities. Thus, the ARPP values decreased. This same effect has been shown to occur in soybeans (Hardy et al., 1972)

Table 4. Means of acetylene reduction rates per plant (ARPP) by date in the first sampling year

Date	Plant density			Cutting management ^a			Cultivar	
	Low	Medium	High	No-cut	3-cut	4-cut	Agate	WL 318
-----μ moles ethylene hour/plant-----								
June 4	1.27	.62	.51**	.76	.75	.88	.86	.74
11	.30	.18	.13**	.24	.23	<u>.15</u>	.23	.18
19	.14	.08	.04**	.10	<u>.07</u>	- *	.08	.09
22	.21	.16	.12	.14	-	.18	.18	.15
28	.17	.12	.08*	.09	.16	- **	.12	.13
July 5	.11	.07	.06**	.06	-	.10**	.08	.08
12	.16	.10	.06**	.10	.19	<u>.03**</u>	.11	.10
26	.17	.12	.09**	.09	.16	<u>.13*</u>	.13	.13
Aug 2	.12	.08	.05	.10	<u>.07</u>	-	.09	.08
8	.17	.13	.14	.15	-	.14	.18	.12*
15	.13	.09	.07*	.09	.17	<u>.03**</u>	.09	.10
29	.06	.05	.04	.04	.07	<u>.04*</u>	.05	.06
Sept 7	.07	.06	.04*	.04	.08	.06**	.06	.06
14	.07	.05	.05	.10	<u>.04</u>	<u>.03**</u>	.06	.05
Oct 1	.07	.07	.05	.06	.08	.04*	.06	.06

^aValues underscored were samplings taken 6 days after a cutting management had been imposed; - indicates data not taken on these dates.

*,**Significant at the 0.05 and 0.01 levels, respectively, among treatments within a factor, within a date.

Table 5. Means of acetylene reduction rates per plant (ARPP) by date in the second sampling year

Date	<u>Plant density</u>		<u>Cutting management^a</u>			<u>Cultivar</u>	
	Low	High	No-cut	3-cut	4-cut	Agate	WL 318
-----μ moles ethylene/hour/core-----							
April 22	.13	.16	.03	.11	.29**	.21	.07**
May 2	.48	.38	.48	.35	.46	.48	.38
15	.49	.37	.62	.35	.33*	.40	.46
29	.27	.22	.36	.20	.17**	.28	.21
June 4	.07	.04	.08	.07	.02*	.06	.05
11	.14	.12	.24	.04	.11**	.17	.09
20	.09	.08	.09	.07	.09	.09	.07
30	.12	.06**	.07	.12	.08	.10	.08
July 7	.03	.04	.05	.06	.00**	.04	.03
15	.04	.03	.03	.04	.02	.03	.03
22	.03	.02	.02	.02	.04	.03	.02
29	.08	.03	.03	.00	.13	.04	.06
Aug 7	.03	.02	.03	.01	.04	.03	.03
14	.02	.02	.03	.02	.00*	.02	.01
22	.02	.02	.01	.02	.04	.02	.03
Sept 2	.05	.03	.02	.03	.06	.04	.03
11	.03	.04	.04	.02	.05	.04	.03
18	.03	.03	.08	.00	.00**	.04	.02
Oct 7	.05	.03	.06	.04	.02	.03	.05

^aValues underscored were samplings taken 6 days after a cutting management had been imposed.

*,**Significant at the 0.05 and 0.01 levels, respectively, among treatments within a factor, within a date.

and field beans (Graham and Rosas, 1978). It was not determined whether the decrease in ARPP values at the higher plant density was a result of changes in nodule fresh weight or a decline in the activity/nodule.

Effect of cutting management A significant ($P < 0.05$) effect caused by cutting management manifested itself in the second year only (Table 1). One possible explanation for this not occurring the first year is the error involved when dividing the μ moles ethylene/hour/core by the number of plants/core. Note from Table 1 that in the first year as compared to the second year, there were more plants/core at each density as a result of loss of plants over time. Thus, there was more variability that could occur within each density. In the second year, because of the fewer number of plants/core on the average, there would be less variability. The higher degree of variability in the first year caused the ARPP values not to be significantly different as a result of cutting management.

It is interesting to note that of the 15 sampling dates used in the first-year analysis (June 4-October 1), all but four followed the same trend within a sampling date as that calculated for ARPP values resulting from cutting management. For example, on July 12 the highest ARPP value resulted from the three-cut management system, followed by the no-cut and then the four-cut management system (Table 2). This same order of ARPP values resulting from cutting management occurred on

the same date (Table 4). Even though there was greater error involved in determining ARPP values the first year, it appears that decreases in ARPP values are also closely associated with cutting the plants. This is reasoned because of the similarity in trends of the ARPP values and the ARPC values. Further, decline in ARPP values as a result of cutting has previously been shown to occur in alfalfa (Vance et al., 1979) and white clover (Moustafa et al., 1969).

The decline in ARPP values is most pronounced on the sampling dates which follow a harvest by 6 days in this study (Tables 4 and 5). The no-cut management system was associated with higher ARPP values in the second year as compared to the three-cut management system which was so associated in the first year (Table 1). These differences in second-year ARPP values resulting from cutting management are caused by the alfalfa plants within a cutting management which did not reach ARPP values between cuttings which were as high above the ARPP values of the no-cut management system as in the first year. Thus, when averaged over the entire season, ARPP values were higher for the three-cut management system than the no-cut management system during the first year.

Effect of cultivar There were no significant differences in ARPP values as a result of cultivar in either year. Only on August 8 in the first year ($P < 0.05$) (Table 4) and April 22 in the second year ($P < 0.01$) (Table 5) were significant differences in ARPP values obtained due to cultivar.

The difference on August 8 in the first year is probably a result of sampling error. The difference on April 22 of the second year could be explained by the differences in winter-hardiness. Agate has more fall dormancy than WL 318 so that growth resumes earlier in the spring for Agate because of less winter injury. The earlier spring growth of Agate resulted in acetylene reduction resuming earlier in the season.

Effect of sampling date Date of sampling had a highly significant ($P < 0.01$) effect on the ARPP values in both years. Higher ARPP values occurred early in the season, but decreased as the season progressed in both years. In the first year, the highest ARPP value of .80 was recorded on June 4 and a low for the season of .06 occurred on September 14. The highest ARPP value in the second year was .43 on May 15 and a season low of .02 occurred on August 14. This variation is related to stage of growth of the plants. Plants in the second year reached flowering in a shorter period of time compared to the first year. The highest ARPP values were reached during a late vegetative to early bud stage of growth. Thus, in the second year, measurement of ARPP values occurred more often when plants were in a more mature stage of growth which caused lower ARPP values.

A highly significant ($P < 0.01$) plant density x date interaction in the first year is noted (Table 4). Those plants at the low plant density began the season with a higher ARPP value than those plants at the medium or high plant density.

By the end of the season, all densities had equal ARPP values. Thus, those plants in the low plant density had a greater decrease in ARPP values compared to the plants at the medium or high density. Apparently, during the first part of the growing season there was less competition between plants at the low density. Later in the season, competition increased causing a decline in ARPP values.

A significant ($P < 0.05$) cutting management x date interaction arose in the second year only (Table 5). The reason for this is similar to that discussed for the cutting management x date interaction which occurred for ARPC values. The no-cut management system began the season at a rate much lower than the other two cutting management systems but by May 15 the ARPP value of the no-cut management system surpassed the ARPP values of the three-cut or four-cut management systems by nearly twofold. By the latter part of the season, equal ARPP values were noted for all cutting managements in this study except for those samplings taken 6 days following a harvest.

Conclusions

Environment and plant growth stage were shown to have considerable influence on the acetylene reduction rates per core or rates calculated per plant. Evidence has been presented to illustrate the inverse relationship between ARPP

and plants/core. The ARPC, however, was not significantly increased with increasing density. Competition among plants obviously becomes a significant factor influencing acetylene reduction rates as density increases.

The effect of a no-cut, three-cut, or four-cut management system was also studied. Within the first 6 days after cutting, there was a decrease in both ARPC and ARPP values. The initial decrease was followed by an increase in ARPC and ARPP values that resulted in values greater than that of the no-cut management system. In the first year, cutting the alfalfa three times resulted in higher average ARPC and ARPP values over the entire season as compared to the no-cut management system. In the second year, however, the no-cut management system resulted in higher ARPC and ARPP values when taken over the entire season. When averaged over the 2 years, there were no differences in ARPC values resulting from cutting managements imposed.

Because the winterhardy cultivar, Agate, had better stand persistence than the moderately winterhardy cultivar, WL 318, higher ARPC values were recorded for Agate than WL 318. There were more plants/core for Agate. There were no differences in the ARPP values measured for the two cultivars.

From the results obtained in this 2-year study, it appears unlikely that the rate of nitrogen fixation as measured by acetylene reduction can be increased by changing plant populations. Cutting management or cutting itself, based on our

study, indicates that rates of nitrogen fixation may be affected. For example, cutting the alfalfa caused an initial decrease of the acetylene reduction rate within the first 6 days followed by an increase to a level equal to or greater than that of plants left uncut. It can be speculated that more nitrogen would be accumulated in harvested plants on a per unit area basis as compared to plants not harvested, because the decreased acetylene reduction rate continued for only a short time period as compared to the increased acetylene reduction rate which occurred after the initial decrease. There was no difference in the rate of nitrogen fixation on a per plant basis of the two cultivars used in this study. This does not conclude, however, that rates of nitrogen fixation could not be changed by selection of a different cultivar. Further study is merited to more clearly answer this question.

Even though no differences in nitrogen fixation rates as measured by acetylene reduction were obtained in this study, it cannot be concluded that there would not be a different response from a nonlegume crop planted in the area with combinations of the treatments imposed on the alfalfa. Whether or not there is a difference in the residual nitrogen available for the following crop needs further research to be clarified.

CHAPTER IV. TOTAL PLANT YIELD AND TOTAL NITROGEN
PERCENTAGE IN ALFALFA (Medicago sativa L.)
AS AFFECTED BY PLANT DENSITY,
CUTTING MANAGEMENT, AND CULTIVAR

Introduction

Recommended practices for the management of alfalfa (Medicago sativa L.) have, in the past, been primarily concerned with the aboveground portion of the plant. Little attention, however, has been given to the total yield and the percentage nitrogen in the entire plant, including the root system. Management practices that affect the aboveground yield and percentage nitrogen will also have an effect on the belowground portion of the plant.

Early studies (Graber et al., 1927; Salmon et al., 1925) have shown a reduction in top yield as the frequency of harvest increases. More recently, Smith (1968) reported greater dry matter yield for alfalfa from three cuttings than from four cuttings when all cuttings were made prior to September 1. A late June, late August, and early October cutting schedule resulted in the highest forage yield as reported by Smith et al. (1968). Hoglund et al. (1974) reported an increase in yield of alfalfa when harvested three times at first flower as compared to cutting five times in the pre-bud stage of growth. Carlton et al. (1968) reported maximum dry matter yields and percentage crude protein in alfalfa harvested between 2 and 45% bloom.

Vance et al. (1979) reported on the effects of shoot removal on the top growth and root growth of alfalfa.

Greenhouse-grown alfalfa plants were subjected to either 70 to 80% shoot removal or left unharvested. The plants were grown from seed and harvested once before treatments were applied at the 1% flower stage of growth. By the 26th day after shoot removal, root weight of unharvested plants increased approximately twofold while the root weight of the plants which were harvested remained nearly unchanged.

Increased forage yield of alfalfa as plant populations increased from 11,955 to 430,372 plants/ha were reported by Jarvis (1962). At populations greater than this, yield was not significantly increased. Root yield taken at the end of the 3-year study showed no differences except the lowest two populations had yields significantly less than the other six populations studied. Bessac (1968) seeded alfalfa in 14.5- and 29-cm rows at rates of 50, 100, or 200 seeds/m of row. If water was not limiting, more forage yield was obtained from the narrower row spacing. Percentage nitrogen was unaffected by seeding treatments imposed. Roufail (1975) reported no difference in forage dry matter yield of alfalfa seeded at six rates ranging from 6.7 to 26.9 kg/ha when seeded in 15-cm rows or broadcast. Yield was significantly less at row spacings of 30 or 45 cm as compared to a 15-cm row spacing. It was also reported that plants at densities of 75 plants/ m^2 had tap roots with a top diameter of 4 cm compared to

plants at densities of 400 plants/m² having a 1-cm diameter tap root. Marten et al. (1963) reported that an alfalfa-bromegrass stand containing 21 to 129 alfalfa plants/m² resulted in no significant differences in yield or percentage protein.

Stickler and Johnson (1957) reported that a nonhardy cultivar of alfalfa yielded more than a hardy cultivar under three different cutting management systems. A rapid-growing Indian cultivar of alfalfa had a lower percentage nitrogen in the root and top material but had a higher dry matter yield than an Australian cultivar, as reported by Sharma et al. (1973).

Studies reported in the literature which discuss the effect of plant density, cutting management, and cultivar do not generally report the effects on total plant yield or nitrogen percentage of roots. We questioned the effect of plant density, cutting management, and cultivar on the total plant dry matter yield and percentage nitrogen of the total plant. It was the objective of the study reported herein, therefore, to determine the effects of plant density, cutting management, and cultivar on the forage dry matter yield, percentage nitrogen in the forage, nitrogen removed in the forage, root dry matter yield, percentage nitrogen in the roots, and nitrogen accumulation in the roots.

Materials and Methods

Experimental design, location, and treatments

The experiment was located on a Webster silty clay loam soil classified as a fine loamy, mixed mesic typic Haplaquoll at the Iowa State University Agronomy and Agricultural Engineering Research Center 13 km west of Ames. The site had been summer-fallowed just prior to seeding on August 8, 1978. Soybeans had been grown in 1975 and 1977 and grain sorghum was planted on this site in 1976.

Plot size was 2.44 x 6.40 m with 13 rows per plot spaced at 20.3 cm. Plots were separated on all sides by alleyways .91 m wide. Three treatment variables were used in this study making a total of 18 treatment combinations. Two cultivars, three seeding rates, and three cutting managements comprised the treatment combinations. The fall dormant, winterhardy cultivar, 'Agate', (Barnes and Frosheiser, 1973) and the moderately fall dormant, moderately winterhardy cultivar, 'WL 318', (Beard and Kawaguchi, 1978) were the two cultivars used in this study. To insure adequate infection, seed was inoculated with a commercial strain of Rhizobium just prior to seeding. Seeding rates were 6.7 (low), 13.5 (medium), and 26.9 (high) kg/ha. Rows were planted in a north-south direction with Planet Jr., single-row planters. Cutting management systems were: (1) no cut, (2) three cuts, and (3) four cuts. There were three replications, arranged in

a randomized complete block design.

All plots received 186 kg/ha K_2O in the fall of 1978 prior to seeding. A split application was applied in 1979 and 1980 with the first application being applied in early spring and the second after the harvest of the first cutting of the three-cut management system. All plots received fertilizer at the same time. Rates applied were 320 kg/ha of K_2O in 1979 and 300 kg/ha in 1980. On June 5, 1979, 90 kg/ha P_2O_5 was applied.

Malathion was applied as needed to control insects, primarily leaf hoppers, during both growing seasons.

Yield and nitrogen percentage: Aboveground

Harvesting Dry matter yield was determined by harvesting to a height of 7.6 cm a .91- x 5.8-m strip in 1979 and a .91- x 4.6-m strip in 1980. Harvest yield was taken from rows 9 through 12 from the east side of each plot. Under the three-cut management system, the first harvest was at approximately 1/10 bloom, with the remaining harvests being equally spaced throughout the remainder of the season. Harvest dates were June 12, July 27, and September 8 in 1979 and June 5, July 23, and September 12 in 1980. The four-cut management system was first harvested at the late-bud stage. Subsequent harvests were equally spaced throughout the remainder of the season. Harvests were made on June 5, July 6, August 9, and September 8 in 1979 and May 29, July 1, August 8, and September 12, 1980. The last harvest in both cutting management systems

was approximately 4 weeks before the first frost of -2.2°C in the fall. The entire plot was harvested at a uniform height, after removing the portion for measuring yield. Subsamples, approximating 800 g in weight, were taken for moisture determination and micro-Kjeldahl analysis.

Laboratory procedure Subsamples in cloth bags were dried at 60°C for 48 hours in a forced-air oven. Percentage dry matter was calculated following the recording of dry weight.

Samples were ground in a Wiley mill using a 1-mm screen. The ground material was thoroughly mixed from which a sample was taken to be used in the determination of total nitrogen by the micro-Kjeldahl method (Bremner, 1965). Ground samples were dried for 24 hours at 65°C prior to weighing a 0.1-g sample used in the determination of total nitrogen.

Yield and nitrogen percentage: Belowground

The location of rows within plots used for root sampling is discussed under plant density. Roots were dug by hand and any excess soil was gently removed by shaking the roots. Residual soil was removed by soaking in water for 12 hours. A 61- x 40.6-cm area was used for collection of the roots. In both years, as much as possible of the root was removed. After removing roots from the soil, all roots were clipped to a uniform length of 20.3 and 30.5 cm in 1979 and 1980, respectively. These lengths included 7.6 cm of the plant above-

ground. Thus, root sampling depth was 12.7 and 22.9 cm in 1979 and 1980, respectively. The shallower depth in 1979 is a result of a less well-developed root system. Thus, the roots severed more readily resulting in a shallower sampling depth compared to the second year. Roots were deposited in cloth bags prior to drying for 48 hours at 45°C in a forced-air oven before weighing to determine dry matter production. Determination of total nitrogen was done in a manner similar to that described previously for top growth.

Plant density

Data for mean number of alfalfa plants/m of 20.3-cm spaced rows are presented in Table 6.

The first stand-density count was made 8 weeks after seeding in the fall of 1978. Stand counts were made again on October 8, 1979 and September 12, 1980.

For the October 1978 count, the plants in four 30.5-cm sections of row randomly selected were counted. Thus, the success of initial establishment was ascertained. This count indicated that the low plant density had a significantly ($P < 0.05$) lower number of plants/m row than the medium or high density. There was no significant difference between the number of plants/m row in the medium or high plant densities (Table 6).

To assess stand density following summer harvests of both years, two 61-cm sections of adjacent rows located at the

Table 6. Mean number of alfalfa plants/m of 20.3-cm spaced rows in the fall of three years^a

Treatment	1978	1979	1980
-----plants/m-----			
Plant density			
Low	23.4a	19.9a	11.5a
Medium	43.0b	33.9b	<u> </u> ^b
High	46.4b	40.0b	17.2b
Cutting management			
No-cut	37.7	26.8	14.2
3-cut	35.8	32.5	15.5
4-cut	39.4	34.6	13.3
Cultivar			
Agate	39.6	34.4a	14.3
WL 318	35.6	28.2b	14.4

^aMeans within a column within a treatment followed by different letters are significantly different at the .05 level of probability. Those without letters are not significantly different.

^bData not taken.

southwest corner of each plot were used for this sampling. A spade was used to remove the plants from the soil. After removal of the roots from the soil, the number of plants was noted and recorded. These roots used in this stand density determination were collected for use in determining dry matter root yield described previously.

Stand density counts in the fall of 1979 indicated little difference in the number of plants/m row between the medium and high seeding rates (Table 6). Because there was little

difference in the number of plants/m row, it would be unlikely that any differences in yield or percentage nitrogen would result from the medium and high plant densities. For this reason, stand density counts were not determined for the medium density in the fall of 1980. The plots originally seeded at the medium plant density were harvested for forage dry matter yield and percentage nitrogen but root dry matter yield and percentage nitrogen was not determined on these plots.

There was a significant difference ($P < 0.05$) in plant density between the two cultivars in 1979 (Table 6). This difference was very small. Thus, any differences in forage or root dry matter yield, or percentage forage or root nitrogen cannot be attributed to differences in plant density of the two cultivars. There was no difference in the densities of the cultivars following summer harvests in the second year.

Results and Discussion

Data will be presented and discussed in two sections: (1) aboveground plant analysis and (2) belowground plant analysis. The aboveground plant analysis consists of forage dry matter yields, percentage nitrogen of the forage, and nitrogen removed in the forage. The belowground plant analysis consists of root dry matter yields, percentage nitrogen in the roots, and nitrogen accumulation in the roots.

Aboveground plant analysis

Forage dry matter yield, percentage of nitrogen, and calculated nitrogen removal data are presented in Table 7. Figures 7, 8, and 9 illustrate these data. Additional tables are located in the Appendix.

Analyses of variance were computed each for forage dry matter yield, percentage nitrogen, and nitrogen removal within each year and over years. The analyses of variance within each year for all data were computed without using the effect due to date of harvest included in the model. This model was utilized because it was not of interest to evaluate the effect of plant density and cultivar within a single harvest date.

Forage dry matter yield No significant differences were noted among dry matter yields resulting from plant density in either year or in the combined-year analysis (Table 7). It can be noted, however, that in the first year of this study, dry matter yields from the low plant density were less than those from the high or medium density. This difference was only significant at $P < 0.10$. These results are in agreement with those of Roufail (1975) for alfalfa seeded in 15-cm rows reporting no significant differences in yield as a result of seeding rate. It has also been reported (Jarvis, 1962) that plant density has little effect on yield at populations greater than 43 plants/m². It is generally found that yield is affected little by differences in plant populations. It is likely that as plant population of alfalfa increases, there

Table 7. Mean forage dry matter yields, percentage nitrogen, and forage nitrogen removal of alfalfa in 2 years and combined years averaged over other treatments^a

Treatment	Yield			Nitrogen			Forage N removal		
	Year 1	Year 2	Comb.	Year 1	Year 2	Comb.	Year 1	Year 2	Comb.
	-----MT/ha-----			-----%-----			-----kg/ha-----		
Plant density									
Low	10.7	13.0	11.9	3.30	3.10	3.20	338	396	357
Medium	11.3	12.7	12.0	3.25	3.13	3.19	349	390	369
High	11.3	13.0	12.1	3.27	3.12	3.20	350	398	374
Cutting management									
3-cut	11.9a	13.5a	12.7a	3.03a	2.88a	2.96a	355a	385	370
4-cut	10.2b	12.3b	11.3b	3.46b	3.29b	3.38b	337b	404	370
Cultivar									
Agate	11.2	13.2	12.2	3.32	3.15	3.23a	352	406a	379a
WL 318	11.0	12.6	11.8	3.23	3.09	3.16b	340	383b	361b

^aMeans within a column within a treatment followed by different letters are significantly different at the .05 level of probability. Those without letters are not significantly different.

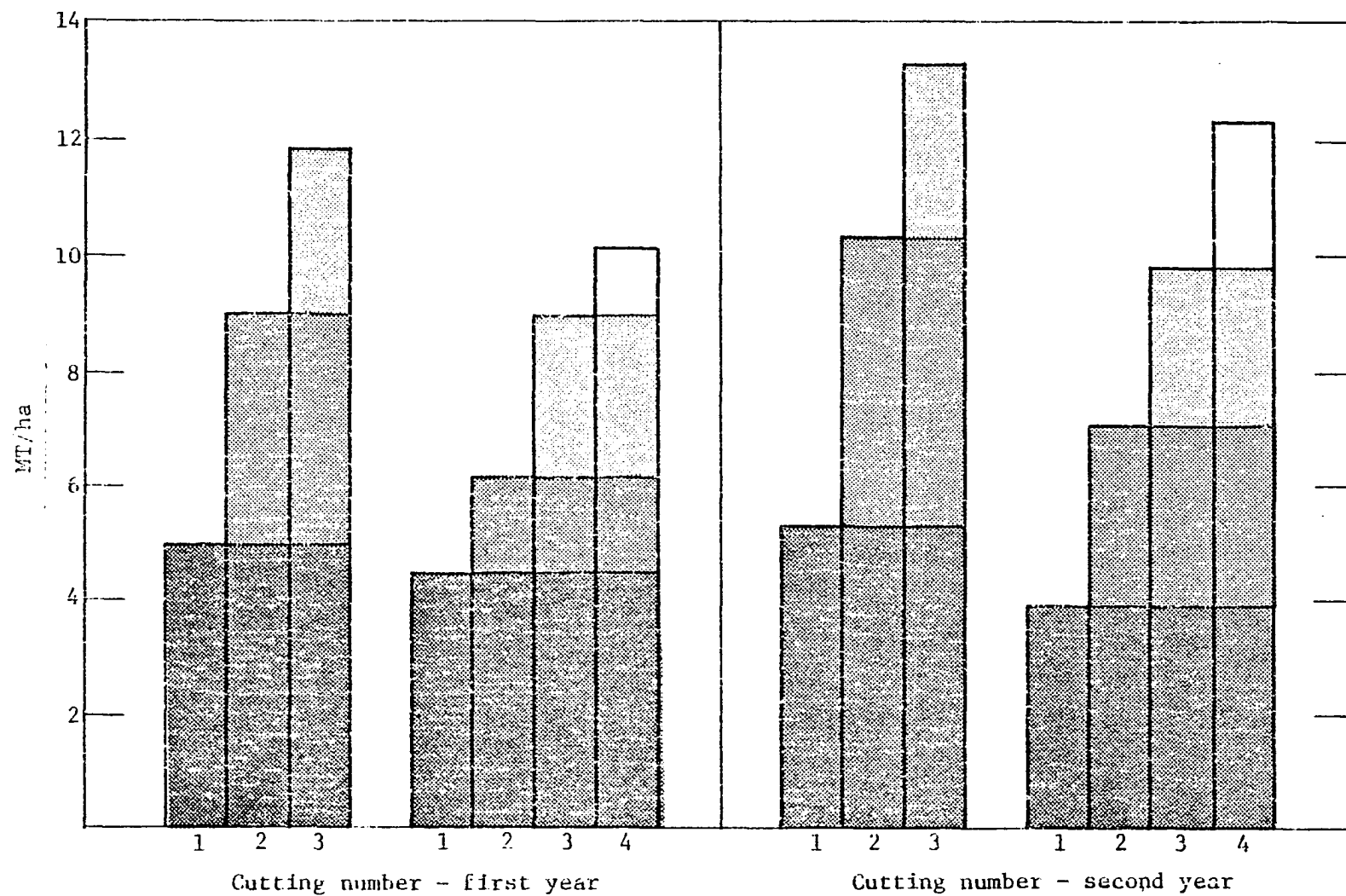


Figure 7. Accumulated forage dry matter yields as affected by cutting management in the first and second year

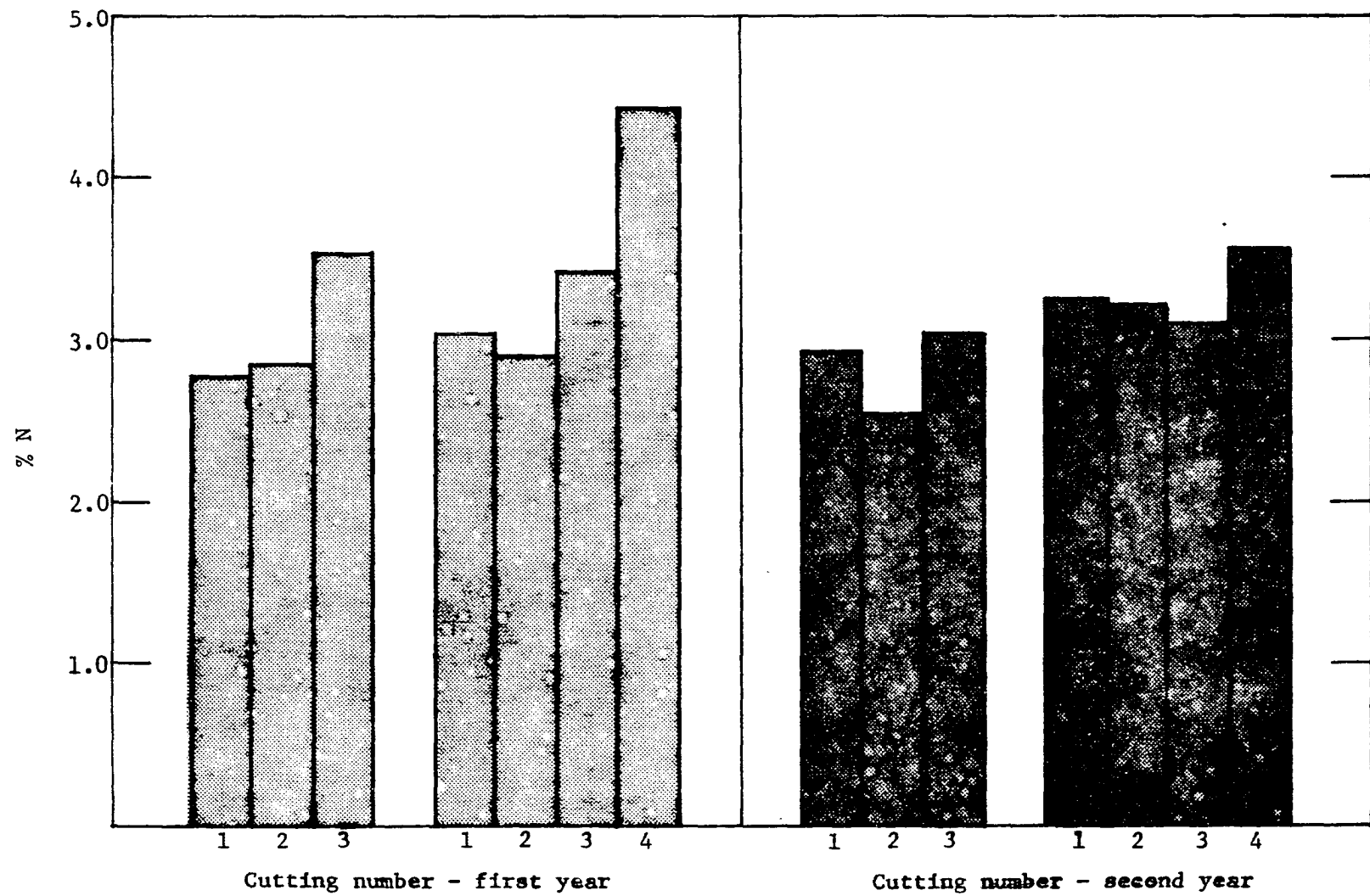


Figure 8. Percentage nitrogen in the forage dry matter as affected by cutting management in the first and second year

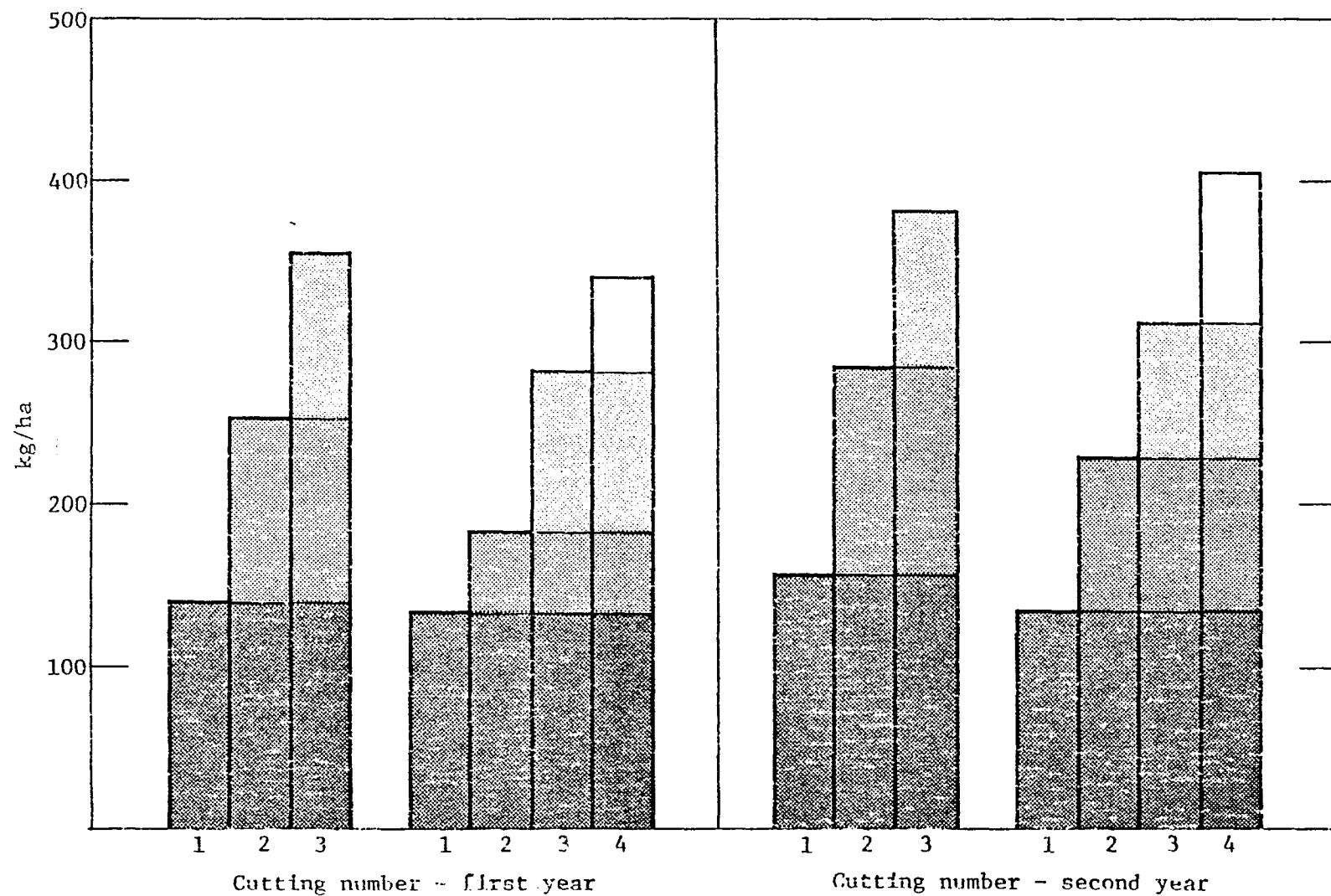


Figure 9. Accumulated forage nitrogen removal as affected by cutting management in the first and second year

is increased competition which results in a lower amount of dry matter produced per plant (Marten et al., 1963). Thus, forage yield is usually not affected.

Differences in yield resulting from cultivar were significant at the 0.10 level of probability in the second year and in the combined-year analysis (Table 7). The winterhardy cultivar, Agate, yielded slightly more than the moderately winterhardy cultivar, WL 318. This is in agreement with data of Porter and Reynolds (1975) who reported that nonwinterhardy cultivars of alfalfa yielded less than the winterhardy cultivars.

There was a highly significant ($P < 0.01$) difference in forage dry matter yield as a result of cutting management in both years and in the combined-year analysis. In both years, more forage was harvested under the three-cut management system (Table 7 and Figure 7). Cutting the alfalfa three times at a later stage of growth compared to four harvests resulted in a higher yield and is in agreement with results reported by Hoglund et al. (1974). Smith (1968) has reported higher forage yields in alfalfa from three cuttings prior to September 1 as compared to four cuttings. Three cuttings have also been shown to be advantageous over two cuttings of alfalfa (Fuess and Tesar, 1968).

In the first year, there was a highly significant ($P < 0.01$) cutting management x cultivar interaction. The winterhardy cultivar, Agate, yielded 12.4 MT/ha compared to 11.5

for WL 318, a moderately winterhardy cultivar, when harvested three times. When harvested four times, however, Agate yielded 10.0 MT/ha compared to 10.5 for WL 318. Agate has slower regrowth so yield is decreased when cut more frequently as compared to WL 318. This cutting management x cultivar interaction was also significant ($P < 0.05$) in the combined-year analysis.

Yields obtained the second year were significantly ($P < 0.01$) higher than in the first year (12.9 vs. 11.1 MT/ha, respectively) (Figure 7). The winter after establishment was colder than normal. Also, the growing season the first year was characterized by below normal temperatures with above normal precipitation compared to above normal temperatures and below normal precipitation during the second growing season. The winter between the two harvest years was generally warmer than normal (see Appendix for exact data on weather conditions). The alfalfa, however, must have been better established the second year, resulting in a higher yield even with the abnormal weather conditions. Subsoil moisture must have been in adequate supply to meet the needs of the alfalfa in the second year when precipitation was below normal.

Forage percentage nitrogen During the 2 years and in the combined-year analysis, forage harvested from the four-cut management system analyzed higher in percentage nitrogen ($P < 0.01$) (Table 7 and Figure 8). Forage harvested from the four-cut management system was of earlier growth stage and

thus of higher percentage nitrogen.

Percentage nitrogen was significantly ($P < 0.01$) lower the second year as compared to the first year (3.12 vs. 3.27%, respectively). Notes on plant growth showed that plants reached flowering earlier in the second year than in the first year. All but the first harvest in each year was on a calendar-day schedule (elapsed days). In the second year, plants were usually more mature at each harvest as compared to the first year. This would account for the lower percentage nitrogen.

In the combined-year analysis, there was a highly significant ($P < 0.01$) difference in percentage nitrogen resulting from cultivars (Table 7). Considered on the individual years, each year Agate had a higher, but nonsignificant, percentage nitrogen. Even though the difference is small, it can account for a considerable amount of nitrogen in the forage. For example, if both cultivars had yielded 12 MT/ha, there would have been 388 kg nitrogen/MT in the forage of Agate compared to 379 in WL 318. Going one step further to show the importance of this small difference, with the same yield as above, on 20 ha of land growing these alfalfa cultivars, there would have been 180 kg more nitrogen removed in the forage of Agate as compared to WL 318.

Forage nitrogen percentage was not affected by plant density (Table 7). These results are in agreement with results of other researchers (Jarvis, 1962; Bessac, 1968) who

reported that plant populations have little effect on the nitrogen percentage of alfalfa.

Forage nitrogen removal In the first year, the three-cut management system resulted in a significantly higher ($P < 0.05$) amount of nitrogen removed per hectare in the forage (Table 7 and Figure 9). Even though there was a higher percentage nitrogen in the forage from the four-cut management system, the larger forage yield of the three-cut management system offset this effect. These results do not agree with those reported by Hoglund et al. (1974) where alfalfa yielded more with later cutting, yet showed no difference in total nitrogen removed. In the second year of the study reported here, more nitrogen removal resulted from the four-cut management system ($P < 0.10$) (Table 7 and Figure 9).

This difference in nitrogen removal each year caused a highly significant ($P < 0.01$) cutting management x year interaction. From Figures 7 and 9 note that the main cause of a lower nitrogen removal in the four-cut management system the first year is caused by the low forage yield. Had the second and fourth harvest of the four-cut management system the first year been as large as it was the second year, there would have been more nitrogen removed in the four-cut management system in both years. It is difficult to attribute the low yield of the second and fourth harvests in the four-cut management system the first year to weather conditions alone. Temperatures during the month in which most of the growth

prior to the second and fourth harvests occurred were within 0.3°C of normal in all months except August of the second year in which temperatures for the month averaged 1.0°C above normal. Precipitation was above normal in June the first year and below normal in June the second year. There was above normal precipitation in August of both years which was the month prior to the fourth harvest (see Appendix for exact weather conditions). From these weather conditions, it appears that the alfalfa was well-established the second year, and thus, it could withstand the below normal precipitation which occurred in June, without decreasing the forage yield.

When averaged over both years, there was no difference in the amount of nitrogen removed in the forage as a result of cutting management (Table 7). From these data, it appears that the amount of nitrogen removed in the forage is more dependent on the dry matter yield than on the percentage nitrogen of the forage. This is reasoned because percentage nitrogen was lower the second year yet more nitrogen was removed.

There was a highly significant ($P < 0.01$) cutting management x cultivar interaction for total nitrogen removed in the first year. This same interaction was significant at $P < 0.10$ in the combined-year analysis. Recall that there was a cutting management x cultivar interaction for yield. The interaction related to nitrogen removal is explained by the forage yield difference as discussed previously. Agate yielded 372

and WL 318 yielded 338 kg/ha of nitrogen under the three-cut management system as compared to 332 and 342 kg/ha for Agate and WL 318, respectively, under the four-cut management system in the first year.

In the second year and combined-year analyses, a significantly ($P < 0.05$) larger amount of nitrogen was removed in the forage of Agate. This same difference also appeared the first year but was only significant at $P < 0.10$ (Table 7). The larger amount of nitrogen removed in the forage of Agate can be attributed mainly to the higher forage dry matter yield. This again suggests that the total amount of nitrogen removed in the forage varies more from dry matter yield differences than from percentage nitrogen differences.

When averaged over all treatments, 346 and 395 kg/ha nitrogen were removed in the first and second year, respectively. The difference was highly significant ($P < 0.01$).

There was no significant difference for forage nitrogen removal as a result of plant density in either year or in the combined-year analysis.

Belowground plant analysis

Data for root dry matter yield, percentage nitrogen in the roots, and nitrogen accumulation in the roots were each analyzed in each year, and data are presented in Tables 8, 9, and 10. A combined-year analysis was not computed because date of sampling was different each year and depth of sampling

Table 8. Mean root dry matter yields, percentage nitrogen, and root nitrogen accumulation of alfalfa in 2 years averaged over other treatments^a

	<u>Yield</u>		<u>Nitrogen</u>		<u>N accumulation</u>	
Treatment	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
	----MT/ha-----		-----%-----		----kg/ha-----	
Plant density						
Low	3.1	4.0	2.23	2.20	71	88
Medium	3.3	-	2.26	-	77	-
High	3.2	3.9	2.26	2.28	72	88
Cutting management						
No-cut	3.9a	3.8a	2.62a	2.35a	102a	90a
3-cut	3.3b	4.8b	2.06b	2.24ab	68b	106a
4-cut	2.4c	3.2a	2.06b	2.14b	50c	68b
Cultivar						
Agate	3.3	4.0	2.23	2.19	76	88
WL 318	3.1	3.8	2.24	2.29	70	88

^aMeans within a column within a treatment followed by different letters are significantly different at the .05 level of probability. Those without letters are not significantly different.

was greater in the second year.

Root dry matter yield

In both years, there was a highly significant ($P < 0.01$) difference in root dry matter yields resulting from cutting management. Less root yield was obtained under the four-cut management system in both years (Table 8). Note that in the first year, a difference of 0.6 MT/ha was significant ($P < 0.05$), but in the second year the least significant difference at the

Table 9. Root dry matter yields, root percentage nitrogen, and root nitrogen accumulation as affected by plant density, cultivar, and cutting management in the first year

		Yield			Nitrogen			N accumulation		
Plant density	Cultivar	Cutting management			Cutting management			Cutting management		
		No-cut	3-cut	4-cut	No-cut	3-cut	4-cut	No-cut	3-cut	4-cut
		-----MT/ha-----			-----% N-----			-----kg/ha-----		
Low	Agate	4.14	3.22	2.33	2.54	1.93	2.16	105	63	51
	WL 318	3.73	3.38	1.97	2.67	2.02	2.04	99	68	40
Medium	Agate	4.34	3.29	2.32	2.77	2.11	2.01	120	69	46
	WL 318	3.72	3.52	2.72	2.52	2.06	2.07	96	73	56
High	Agate	4.57	3.22	2.44	2.58	2.15	2.04	117	69	50
	WL 318	2.89	3.04	2.86	2.65	2.10	2.01	76	64	58

Table 10. Root dry matter yield, root percentage nitrogen, and root nitrogen accumulation as affected by plant density, cultivar, and cutting management in the second year

		Yield			Nitrogen			N accumulation		
Plant density	Cultivar	Cutting management			Cutting management			Cutting management		
		No-cut	3-cut	4-cut	No-cut	3-cut	4-cut	No-cut	3-cut	4-cut
		-----MT/ha-----			-----% N-----			-----kg/ha-----		
Low	Agate	3.57	5.55	3.11	2.29	2.15	2.00	82	119	62
	WL 318	3.69	5.28	2.80	2.33	2.38	2.04	86	122	57
High	Agate	4.53	4.03	3.35	2.36	2.12	2.21	106	86	72
	WL 318	3.57	4.28	3.45	2.41	2.29	2.31	86	98	79

0.05 level of probability was 0.9 MT/ha. One explanation is the number of samples used to determine a cutting management mean which, in the second year, did not include the medium plant density.

In the first year, greater root yield was produced under the no-cut management system. This did not, however, hold true the second year in which the largest yield was obtained under the three-cut management system. Note from Table 8 that root yield increased 1.5 MT/ha (3.3 to 4.8) under the three-cut management system and 0.8 (2.4 to 3.2) under the four-cut management system compared to a decrease of 0.1 (3.9 to 3.8) under the no-cut management system from the first to the second year. This suggests that cutting the alfalfa causes an increase in root growth compared to leaving the alfalfa uncut. This is possibly related to stage of growth of the plants. Under the three-cut management system the second year, plants reached full flower prior to the second and third harvests. The plants under the four-cut management system reached full flower only once during the growing season the second year. Thus, those plants under the three-cut management system had time to utilize some of the photosynthates for root growth causing a greater increase in root yield over that of the four-cut management system. Even though the plants were not removed from the no-cut management system, these plants must not have been as actively growing as compared to those plants which were harvested three or four

times during the second growing season.

A cutting management x plant density interaction for root yield was significant ($P < 0.10$) the second year (Table 10). There was more root yield resulting from the low plant density as compared to the high plant density under the three-cut management system. Competition must have been less and, in combination with the cutting management system, this resulted in a more vigorous root system and a higher yield.

There were no significant differences in root yield as a result of plant densities in either year. This study is in agreement with Jarvis (1962) who found no differences in root yield of alfalfa as a result of plant populations.

The winterhardy cultivar, Agate, yielded 0.2 MT/ha root dry matter more each year than the moderately winterhardy cultivar, WL 318, but this difference was not statistically significant (Table 8).

Root percentage nitrogen The no-cut management system had a higher root percentage nitrogen, $P < 0.01$ for the first year and $P < 0.05$ for the second year (Table 8). In the second year, the percentage root nitrogen of the no-cut management system was not significantly higher than that of the three-cut management system. The higher percentage nitrogen resulting from the no-cut management system is associated with forage not being removed as less of the root nitrogen would be mobilized to the top growth. In this study, cutting management (three vs. four cuts) had no significant effect on the

root percentage nitrogen (Table 8).

In the second year, WL 318 had a higher ($P < 0.10$) root percentage nitrogen than Agate (Table 8). This difference did not occur the first year. No clear explanation is apparent for the difference in root percentage nitrogen as a result of cultivar the second year.

No significant differences in root percentage nitrogen resulted from the plant densities studied in either year.

Root nitrogen accumulation Highly significant differences ($P < 0.01$) in root nitrogen accumulation resulted from cutting management in both years (Table 8). In the first year, a very obvious trend developed. More nitrogen accumulated in the roots with a decreased number of cuttings. This trend did not manifest itself the second year largely because of the high root dry matter yield in the three-cut management system. When averaged over the 2 years, 96, 87, and 59 kg/ha of nitrogen were accumulated in the roots of the no-cut, three-cut, and four-cut management systems, respectively. The same trend apparent in the first year for root nitrogen accumulation was evident when averaged over the 2 years. Recall, however, that the root yield increased more under the three-cut and four-cut management systems than the no-cut management system. If this trend in root yield were to continue for another year, the amount of root nitrogen accumulation would be higher under the three-cut management system rather than under the no-cut management system. A trend that

developed from the first to the second year is that root nitrogen accumulation increased from cutting and decreased when plants are left uncut.

There was a significant ($P < 0.05$) plant density x cutting management interaction for root nitrogen accumulation in the second year, but not in the first year (Tables 9 and 10). Note in the low plant density, the large values for nitrogen accumulation under the three-cut management system compared to those in the high plant density. It appears that nitrogen accumulation is related to root yield more than root percentage nitrogen. Those plants in the low density developed a more vigorous root system and in combination with the three-cut management system yielded more root dry matter. This increased root yield resulted in a higher amount of root nitrogen accumulation.

There were no significant differences in root nitrogen accumulation as a result of plant density or cultivar in either year (Table 8). Although Agate had a greater root yield than WL 318, the higher percentage nitrogen of WL 318 offset this effect, resulting in equal nitrogen accumulation.

Conclusion

Effects of plant density, cutting management, and cultivar of alfalfa on forage and root dry matter yield, forage and root percentage nitrogen, forage nitrogen removal, and root nitrogen accumulation were studied.

In the 2 years of the study, no significant differences in forage or root dry matter yield, forage or root percentage nitrogen, forage nitrogen removal, or root nitrogen accumulation resulted from the densities used in this study.

Cutting the alfalfa three times as compared to four times per season resulted in a larger ($P<0.01$) forage dry matter yield in both years. A higher ($P<0.01$) forage percentage nitrogen resulted from the four-cut management system in both years of the study as compared to the three-cut management system. Forage nitrogen removal was significantly ($P<0.05$) higher under the three-cut management system in the first year and neared significance in favor of the four-cut management system in the second year. When averaged over the 2 years, there was no difference in the forage nitrogen removal. Root dry matter yield and root nitrogen accumulation were significantly ($P<0.01$) higher under the no-cut management system the first year and under the three-cut management system the second year. Root percentage nitrogen was higher under the no-cut management system, $P<0.01$ the first year and $P<0.05$ the second year.

When averaged over both years, Agate, a winterhardy cultivar, had a significantly ($P<0.01$) higher forage percentage nitrogen than WL 318, a moderately winterhardy cultivar. There was a significantly ($P<0.05$) greater amount of nitrogen removed in the forage of Agate compared to WL 318 in the second year and when averaged over both years. Forage dry

matter yield was not significantly affected by differences in cultivars. There were no differences in root dry matter yield, root percentage nitrogen, or root nitrogen accumulation as a result of cultivar.

In considering the amount of nitrogen removed in the forage or accumulated in the roots, each appears to be favored by a different cutting management system according to the results of this study. In the second year of the study, it was shown that more nitrogen was removed under a four-cut management system as compared to more root nitrogen accumulation under a three-cut management system. Because of this inverse relationship, nearly equal amounts of total plant nitrogen were produced whether the plant was harvested three or four times during the year.

Selection of a winterhardy cultivar may result in more nitrogen removal than would be removed in the forage of a less winterhardy cultivar, according to results obtained in this study. A winterhardy cultivar may accumulate slightly more nitrogen in the roots, as compared to a moderately winterhardy cultivar.

It seems apparent that the densities used in this study have no effect on total plant yield or total plant percentage nitrogen.

CHAPTER V. CONVERGENCE OF DATA PRESENTED AND IMPLICATIONS IN ALFALFA MANAGEMENT

Before the advent of commercial fertilizers, crop producers relied heavily on forage legumes such as alfalfa for nitrogen. For several decades following, crop producers have enjoyed the use of relatively inexpensive nitrogen fertilizer. Since approximately 1973, the cost of commercial nitrogen fertilizer has begun to increase steadily (Munson, 1978). It should be noted, however, that economic returns from nitrogen fertilizer use is favorable inasmuch as the cost per pound of nitrogen as compared to the selling price of one bushel of corn remains at a ratio of approximately 20:1. Should this ratio decrease from this, coupled with a possible scarcity of fossil fuel used in nitrogen production, alfalfa would become an increasingly important source of nitrogen in the cropping sequence.

That the nitrogen is particularly important in corn production has been shown in long-term studies. Reported have been decreases in corn yields when grown without crop rotation or application of nitrogen fertilizer (Haynes and Thatcher, 1955). Soil nitrogen levels will decrease under continuous corn if nitrogen fertilizer is not applied (Anderson and Peterson, 1973).

Nitrogen Benefit from Harvested Alfalfa

It has been well-established that alfalfa in the cropping sequence will improve corn yields following alfalfa in the absence of applied commercial nitrogen fertilizer (Triplett, 1962; Mitchell and Teel, 1977). In our study, an objective was to determine if there was an advantage for harvesting alfalfa three times versus four times. Also, what benefit could be imputed to alfalfa for a nonlegume crop following?

Based on the results of our study, there was a total of approximately 370 kg/ha/year of nitrogen removed in the forage when harvested either three or four times during the growing season. Also, after 2 years of growth, there was an additional 106 and 68 kg/ha of nitrogen in the roots and crowns of the alfalfa under a three-cut and four-cut management system, respectively. It has been reported that an amount equal to one-third of that amount of nitrogen removed in the forage is left in the soil as residual nitrogen (Munson, 1978). Using this assumption in the study reported here, there should have been 123 kg/ha nitrogen ($370/3 = 123$) remaining in the roots and soil under either the three-cut or four-cut management system. The nitrogen measured in the roots and crowns of this experiment does not, however, account for this greater amount. Thus, it could be assumed that the remaining nitrogen expected was either in the soil or in root material not sampled.

That a substantial portion of this residual nitrogen is

available for a following corn crop under midwest United States conditions can be ascertained from data reported by Higgs et al. (1976). They reported that first-year corn following 2 years of alfalfa yielded 82.9 q/ha as compared to 86.0 q/ha for first-year corn following alfalfa which also received 84.1 kg/ha of commercial nitrogen fertilizer.

Nitrogen Benefit from Unharvested Alfalfa

The study reported here included a no-cut management system. When left unharvested, alfalfa continued to reduce acetylene (fix nitrogen) throughout the entire growing season based on data from our study. At the end of the second year, 90 kg/ha nitrogen had accumulated under the no-cut management system in the surface 22.9 cm of soil and the 7.6 cm of plant material aboveground (roots and crowns). This compared to 106 and 68 kg/ha nitrogen under the three- and four-cut management system, respectively. Continuing further regarding the no-cut management system, the amount of forage production aboveground under the no-cut management system cannot be ascertained. Can it be assumed that the forage aboveground contains approximately as much nitrogen as was removed under the three- and four-cut management systems? Bolton et al. (1976) determined that corn following 1 or 2 years of unharvested alfalfa yielded more than continuous corn when both had received 6.6 kg/ha nitrogen at seeding and 110 kg/ha nitrogen as a sidedressing. Higgs et al. (1976) reported

higher first-year corn yields following harvested alfalfa as compared to continuous corn. They also reported that after 1 year of harvested alfalfa, second-year corn yields were higher than continuous corn but lower than yields of first-year corn following 1 year of harvested alfalfa. Though data in our study are limited, we feel there could be a beneficial effect on second-year corn yields from not harvesting the alfalfa and plowing it down in the fall.

Comparison of Measurement Techniques

In one part of our study (Chapter III), we measured the rate of nitrogen fixation by use of acetylene reduction. In a second part (Chapter IV), the percentage nitrogen in the plant material was determined by micro-Kjeldahl analysis. These percentages, along with dry matter yield, were used to calculate the amount of nitrogen removed in the forage.

It is of interest to compare the two measurements in distinguishing differences as a result of cutting management. In the first year of our study, more nitrogen was removed in the forage of the three-cut management system as compared to the four-cut management system. This was reversed in the second year. The mean acetylene reduction rate for the season during the first year also was highest for the three-cut management system. Similarly, in the second year, the acetylene reduction rate was higher under the four-cut management system as compared to the three-cut management system. Thus,

from these data it can be seen that the acetylene reduction measurement and the removed calculations (micro-Kjeldahl analysis x yield) were both estimating the effects of cutting the alfalfa on the amount of nitrogen produced.

Management of Alfalfa for Maximum Nitrogen Fixation

Cutting management

The data obtained in this study indicate that those cutting management practices that result in high yield and quality alfalfa should also result in maximum rates of nitrogen fixation.

Acetylene reduction rates indicate that as much nitrogen was being fixed in a no-cut management system as in a system which included cutting. It was also shown that rates of acetylene reduction reflected the amount of nitrogen in the plant. It may be possible to assume that as much nitrogen was produced by the no-cut management system as a system of cutting. Further, it can be assumed that after 1 year, there would be 472 kg/ha (370 from the tops + 102 from the roots = 472) nitrogen under a no-cut management system of alfalfa. Not all of this nitrogen will become available to a subsequent nonlegume crop, at least in the first year. Thus, this decreases the amount of the nitrogen attributable to the alfalfa which could be considered as a gain to the crop producer needing nitrogen in the first year after alfalfa. Gains in corn yields, however, may be realized the second year after a crop

of unharvested alfalfa. At present prices, it is unlikely that a crop producer can afford to produce a crop of alfalfa for the sole purpose of obtaining nitrogen. This is so because of the current prices of land, machinery, and operating expenses.

If top management is used in growing of alfalfa, the crop producer can realize maximum yields and quality as well as having the benefit of the residual nitrogen for a subsequent nonlegume crop (if the forage is utilized).

Cultivar

No significant differences in acetylene reduction rates were obtained in our study as a result of cultivars used. A winterhardy cultivar, however, removed a significantly higher amount of nitrogen in the forage as compared to a moderately winterhardy cultivar. Higher rates of acetylene reduction may result from cultivars which are more winterhardy than the one used in this study.

Plant density

Reasoning from the data in the study reported herein, it is unlikely that more nitrogen can be fixed by altering plant density. It appears that a 13.5 kg/ha seeding rate is sufficient to obtain an adequate stand of alfalfa. Rates above or below this did not change the amount of acetylene reduced on a per unit area basis. No differences were noted in the amount of nitrogen removed in the forage as a result of the

plant densities studied.

Future Research Needs

The study reported here indicated that maximum rates of acetylene reduction occurred in mid-May. If the alfalfa is left unharvested, much lower acetylene reduction rates are observed after this initial peak. It was also shown in our study that acetylene reduction rates decreased as the alfalfa matured. It is not known if this decline in acetylene reduction rate is due entirely to plant maturity or as a result of maturing in combination with some other factor or factors, e.g., moisture, shading.

Further research appears merited to determine more closely the seasonal pattern of acetylene reduction of harvested alfalfa plants. Sampling more often during the growing season could provide the needed data on exact pattern of acetylene reduction as affected by frequency of cutting.

A third point which needs further clarification is the effect of alfalfa on a subsequent nonlegume crop. Several questions arise. Is more residual nitrogen utilized by a corn crop following alfalfa harvested four times as compared to three times? Could a crop producer plow down the alfalfa shortly after it reaches its peak acetylene reduction rate and follow by planting a short season crop which would benefit from the residual nitrogen?

Selection of cultivars is important in the growing of alfalfa. In our study, only slight nonsignificant differences in acetylene reduction rates resulted from the two cultivars studied. A wide range of cultivars varying in winterhardiness should be examined to determine if differences in acetylene reduction rates exist.

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APPENDIX

Table A1. Acetylene reduction rates per core (ARPC) and acetylene reduction rates per plant (ARPP) by date averaged over all treatments in the first and second year

First year (1979)			Second year (1980)		
Date	ARPC	ARPP	Date	ARPC	ARPP
	--- μ moles ethylene/hr-			--- μ moles ethylene/hr-	
April 30	1.59	- ^a	April 22	.39	.14
May 9	3.84	-	May 2	1.43	.43
23	3.99	-	15	1.18	.43
			29	.58	.25
June 4	2.65	.80	June 4	.14	.05
11	.69	.21	11	.27	.13
19	.30	.09	20	.19	.08
22	.59	.16	30	.21	.09
28	.42	.13			
July 5	.28	.08	July 7	.08	.04
12	.34	.11	15	.06	.03
26	.41	.13	22	.06	.03
			29	.08	.05
Aug 2	.26	.08	Aug 7	.06	.03
8	.56	.15	14	.04	.02
15	.31	.10	22	.06	.02
29	.17	.05			
Sept 7	.18	.06	Sept 2	.07	.04
14	.17	.06	11	.07	.04
			18	.04	.03
Oct 1	.20	.06	Oct 7	.08	.04

^aData not taken on these dates.

Table A2. Dry matter top yield as affected by plant density, cultivar, and cutting management in the first year

Plant density	Cultivar	3-cut management				4-cut management				
		Cutting no.				Cutting no.				
		1	2	3	Total	1	2	3	4	Total
		-----MT/ha-----				-----MT/ha-----				
Low	Agate	4.88	3.93	3.14	11.95	4.15	1.61	2.62	1.08	9.46
	WL 318	4.51	3.62	2.83	10.96	4.38	1.74	2.99	1.38	10.49
Medium	Agate	5.47	4.24	3.11	12.82	4.21	2.10	2.61	1.07	9.99
	WL 318	4.86	3.95	3.03	11.84	4.31	2.05	2.95	1.24	10.55
High	Agate	5.31	4.29	2.81	12.41	4.29	2.10	2.91	1.16	10.46
	WL 318	4.97	3.84	2.88	11.69	4.31	2.08	2.89	1.26	10.54

Table A3. Dry matter top yield as affected by plant density, cultivar, and cutting management in the second year

Plant density	Cultivar	3-cut management				4-cut management				
		Cutting no.				Cutting no.				
		1	2	3	Total	1	2	3	4	Total
		-----MT/ha-----				-----MT/ha-----				
Low	Agate	6.00	4.90	3.29	14.19	4.31	2.97	2.68	2.38	12.34
	WL 318	5.17	4.66	3.15	12.98	4.15	3.08	2.95	2.37	12.55
Medium	Agate	5.58	4.58	2.92	13.08	3.77	2.99	2.80	2.50	12.06
	WL 318	5.40	4.67	3.38	13.45	3.81	2.88	2.91	2.52	12.12
High	Agate	5.88	5.29	3.18	14.35	4.47	3.02	2.93	2.69	13.11
	WL 318	4.96	4.89	2.95	12.80	3.70	2.88	2.70	2.44	11.72

Table A4. Percentage nitrogen in the top growth as affected by plant density, cultivar, and cutting management in the first year

Plant density	Cultivar	<u>3-cut management</u>			<u>4-cut management</u>			
		<u>Cutting no.</u>			<u>Cutting no.</u>			
		1	2	3	1	2	3	4
		-----% N-----			-----% N-----			
Low	Agate	2.81	2.99	3.68	3.07	3.00	3.51	4.51
	WL 318	2.71	2.81	3.43	3.01	2.89	3.47	4.34
Medium	Agate	2.79	2.74	3.49	3.06	2.86	3.61	4.41
	WL 318	2.78	2.87	2.32	3.00	2.92	3.19	4.36
High	Agate	2.83	2.81	3.48	3.11	2.97	3.49	4.54
	WL 318	2.69	2.69	3.56	3.09	2.91	3.24	4.37

Table A5. Percentage nitrogen in the top growth as affected by plant density, cultivar, and cutting management in the second year

Plant density	Cultivar	<u>3-cut management</u>			<u>4-cut management</u>			
		<u>Cutting no.</u>			<u>Cutting no.</u>			
		1	2	3	1	2	3	4
		-----% N-----			-----% N-----			
Low	Agate	2.96	2.62	3.09	3.20	3.22	3.19	3.61
	WL 318	2.99	2.64	3.01	3.15	3.29	2.91	3.52
Medium	Agate	2.99	2.57	3.21	3.36	3.30	3.27	3.49
	WL 318	2.98	2.60	2.87	3.33	3.27	3.03	3.55
High	Agate	2.93	2.65	3.17	3.14	3.25	3.15	3.70
	WL 318	2.92	2.69	2.98	3.34	3.11	3.11	3.57

Table A6. Nitrogen removed in the top dry matter as affected by plant density, cultivar, and cutting management in the first year

Plant density	Cultivar	3-cut management				4-cut management				
		Cutting no.				Cutting no.				
		1	2	3	Total	1	2	3	4	Total
		-----kg/ha-----				-----kg/ha-----				
Low	Agate	137.2	117.2	115.6	370.0	126.9	48.7	91.7	48.7	316.0
	WL 318	122.6	101.1	96.9	320.6	131.8	50.6	103.6	59.9	345.9
Medium	Agate	152.3	116.2	109.0	377.5	128.7	59.7	94.0	46.8	329.2
	WL 318	135.3	113.2	103.5	352.0	129.1	60.0	93.9	54.4	337.4
High	Agate	150.2	120.5	97.9	368.6	133.4	62.8	101.7	52.4	350.3
	WL 318	133.6	103.1	102.2	338.9	132.9	60.9	93.7	54.8	342.3

Table A7. Nitrogen removed in the top dry matter as affected by plant density, cultivar, and cutting management in the second year

Plant density	Cultivar	3-cut management				4-cut management				
		Cutting no.				Cutting no.				
		1	2	3	Total	1	2	3	4	Total
		-----kg/ha-----				-----kg/ha-----				
Low	Agate	176.8	126.9	101.8	405.5	137.9	96.2	84.8	86.0	404.9
	WL 318	153.9	122.6	94.3	370.8	130.6	101.6	85.7	83.4	401.3
Medium	Agate	166.9	117.4	93.5	377.8	126.5	99.0	91.6	87.3	404.4
	WL 318	160.3	121.5	96.6	378.4	126.5	94.1	88.2	89.4	398.2
High	Agate	172.4	140.9	100.8	414.1	140.4	98.3	92.2	99.7	430.6
	WL 318	144.8	131.4	87.9	364.1	123.3	89.4	83.9	87.0	383.6

Table A8. Monthly mean temperatures and departures from normal at the experiment location

Month	1978		1979		1980	
	Avg	Dptr	Avg	Dptr	Avg	Dptr
-----°C-----						
January			-14.6	-7.3	-6.2	1.1
February			-11.4	-6.9	-6.6	-2.1
March			0.4	-0.5	0.8	-0.1
April			7.2	-2.5	10.7	1.1
May			15.7	-0.1	17.1	1.3
June			21.1	0.3	20.9	0.2
July			22.4	-0.7	24.8	1.7
August	21.9	-0.3	22.0	-0.2	23.2	1.0
September	20.5	3.2	18.3	1.1	18.9	1.6
October	10.4	-1.6	11.3	-0.6	9.6	-2.3
November	2.1	-0.8	2.3	-0.6		
December	-6.9	-2.7	-0.9	3.2		

Table A9. Monthly mean precipitation and departures from normal at the experiment location

Month	1978		1979		1980	
	Avg	Dptr	Avg	Dptr	Avg	Dptr
-----mm-----						
January			28	6	29	7
February			8	-14	8	-14
March			89	37	11	-41
April			110	29	30	-50
May			123	9	73	-41
June			161	14	100	-47
July			103	16	51	-36
August	100	8	126	34	137	45
September	142	58	65	-18	38	-45
October	19	-36	98	42	36	-20
November	65	36	50	21		
December	16	-8	6	-18		